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FULL-LENGTH ORIGINAL RESEARCH

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Epilepsy-predictive magnetic resonance imaging changes following experimental febrile status epilepticus: Are they translatable to the clinic?

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Summary

Objective: A subset of children with febrile status epilepticus (FSE) are at risk for development of temporal lobe epilepsy later in life. We sought a noninvasive predictive marker of those at risk that can be identified soon after FSE, within a clinically realistic timeframe.

Methods: Longitudinal T_2-weighted magnetic resonance imaging (T_2WI MRI) of rat pups at several time points after experimental FSE (eFSE) was performed on a high-field scanner followed by long-term continuous electroencephalography. In parallel, T_2WI MRI scans were performed on a 3.0-T clinical scanner. Finally, chronic T_2WI MRI signal changes were examined in rats that experienced eFSE and were imaged months later in adulthood.

Results: Epilepsy-predicting T_2 changes, previously observed at 2 hours after eFSE, persisted for at least 6 hours, enabling translation to the clinic. Repeated scans, creating MRI trajectories of T_2 relaxation times following eFSE, provided improved prediction of epileptogenesis compared with a single MRI scan. Predictive signal changes centered on limbic structures, such as the basolateral and medial amygdala. T_2WI MRI changes, originally described on high-field scanners, can also be measured on clinical MRI scanners. Chronically elevated T_2 relaxation times in hippocampus were observed months after eFSE in rats, as noted for post-FSE changes in children.

Significance: Early T_2WI MRI changes after eFSE provide a strong predictive measure of epileptogenesis following eFSE, on both high-field and clinical MRI scanners. Importantly, the extension of the acute signal changes to at least 6 hours after the FSE enables its inclusion in clinical studies. Chronic elevations of T_2 relaxation times within the hippocampal formation and related structures are common to human and rodent FSE, suggesting that similar processes are involved across species.

KEYWORDS
epileptogenesis, febrile seizures, febrile status epilepticus, magnetic resonance imaging, temporal lobe epilepsy

1 | INTRODUCTION

Fever-associated seizures are common, occurring in 2%-5% of children.[1] Normally, they are short and without long-term consequences, but seizures lasting >30 minutes are categorized as febrile status epilepticus (FSE) and are an important risk factor for developing temporal lobe epilepsy.[2-4] Between the FSE and the first spontaneous epileptic seizure, there are years of epileptogenesis called the latent period.[4-7] The latent period can last a decade or more, but currently clinicians are not able to predict which children will develop epilepsy following FSE and which will remain healthy.[8,9] A noninvasive technique to predict epileptogenesis would allow clinicians to appropriately counsel and monitor patients and ideally eventually provide a preventative intervention to those at risk before the first spontaneous seizure occurs.

To reach this goal, we previously reported that early magnetic resonance imaging (MRI) changes can predict epileptogenesis in an immature rat model of experimental FSE (eFSE).[8] When rat pups underwent high-field 11.7-T MRI scans 2 hours after eFSE, a decrease in T_2 relaxation time within the basolateral amygdala (BLA) was predicative of which rats would develop epilepsy. This built on a number of studies in rodents and humans that have found MRI changes in the days, months, and years after eFSE, reflecting long-term changes in the brain following a single episode of FSE.[10-18] In contrast to the very early decrease (2 hours post-eFSE) in T_2 signal reported in Choy et al,[8] studies at later time points and on lower magnetic field scanners reported increased T_2 relaxation times. For example, the FEBSTAT study, a prospective study of FSE in childhood, found that both an initial MRI scan (within 1 week of FSE) and follow-up scans in the months and years following revealed hyperintensity (increased T_2 signal) and volumetric changes in the hippocampus.[10,11] The increased T_2 relaxation time is consistent with our MRI finding in rats at 1 month following eFSE.[12]

This work was undertaken to advance the translatability of the early, predictive MRI signal to the clinic. Specifically, our goals were to: (1) increase the sensitivity and specificity of the prediction of epileptogenesis; (2) increase the time window for imaging evaluation, to allow a clinically relevant interval between the FSE and imaging; and (3) demonstrate that the changes that were observed on a 11.7-T high-field magnetic field scanner can be translated to the clinically available low-field scanners (ie, 3.0 T).

Key Points

- Longitudinal MRI trajectories post-FSE enhance prediction of epileptogenesis
- Predictive MRI changes following FSE persist for at least 6 hours and can also be measured on clinical scanners
- Chronically increased T_2, emblematic of post-FSE changes in children can be observed in FSE-experiencing rats

2 | MATERIALS AND METHODS

All experimental procedures were approved by the University of California, Irvine and Loma Linda University institutional animal care and use committees and conformed to National Institutes of Health guidelines. Sprague-Dawley rats (Envigo, Livermore, California) were maintained in quiet facilities under controlled temperatures and light-dark cycles. Cages were monitored every 12 hours, and the date of birth was considered postnatal day 0 (P0). On P2, litters were culled to 10-11 pups, to encourage consistent development.

2.1 | Induction of eFSE

eFSE was induced as previously described.[8,19-21] Briefly, on P10-11, pups were placed in pairs in a 3.0-L glass container lined with absorbent paper. Pups were subjected to a continuous stream of warm air until behavioral seizures began (3-5 minutes), characterized by sudden loss of motion (freezing), oral automatisms, and forelimb clonus. Hyperthermia (39.0-41.5°C) was maintained for 40 minutes (Cohort 1) or 60 minutes (other cohorts), an increased duration aiming to generate seizures lasting >50 minutes, as suggested by human studies.[10,22] Core temperatures were measured at baseline, seizure onset, and every 2 minutes during hyperthermia. Following eFSE, rats were cooled by placing them on a cool metal surface or under running water and allowed to recover for 15 minutes before returning to their home cage.

The cohorts of rats studied were:

- Cohort 1: Male only control (n = 14) and eFSE rats (n = 19) were serially scanned at 2, 18, and 48 hours at 11.7 T, and underwent continuous electroencephalographic (EEG) studies for up to 10 months. Whereas some data from this cohort have been reported,[8] the analyses presented here are previously unreported (Figure 1).
- Cohort 2: Imaged in vivo at 11.7 T 2 and 6 hours after eFSE (8 controls, 10 eFSE). Both males and females were used and randomly assigned to both groups (Figure 2).
- Cohort 3: Imaged in vivo at 3.0 T 4 and 48 hours after eFSE (7 controls, 9 eFSE). Both males and

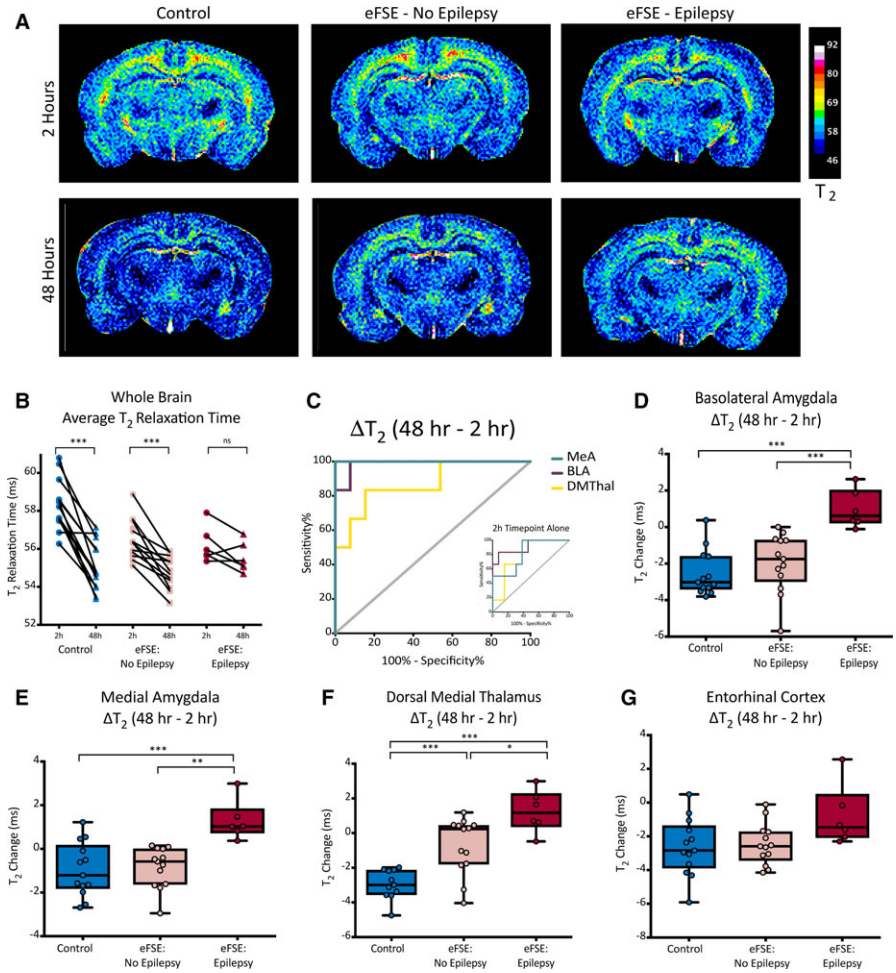

FIGURE 1 The trajectory in T_2 differences (48 vs 2 hours) following experimental febrile status epilepticus (eFSE) is a better predictor of epileptogenesis than single early time point alone. A, Representative pseudocolored 11.7-T T_2 maps of a control rat, an eFSE rat that did not develop epilepsy, and an eFSE rat that went on to develop epilepsy. B, Whole brain T_2 values in individual animals decreased significantly in control and eFSE-NoEpi, but not in eFSE-Epi, (control $t = 7.94$, $df = 12$, $P < 0.001$; eFSE-NoEpi $t = 5.54$, $df = 11$, $P < 0.001$; eFSE-Epi $t = 2.022$, $df = 5$, $P = 0.10$). C, Receiver operating characteristic (ROC) curve of the predictive value of the delta T_2 between 2 and 48 hours of the basolateral amygdala (BLA), medial amygdala (MeA), and dorsal medial thalamus (DMThal; BLA: area under the curve [AUC] = 0.99 ± 0.020, $P = 0.001$; MEA: AUC = 1.00 ± 0, $P = 0.001$; DMThal: AUC = 0.83 ± 0.098, $P < 0.05$; inset: original ROC of 2-hour time point alone, as published in Choy et al[8]; BLA: AUC = 0.91 ± 0.08, $P = 0.005$; MEA: AUC = 0.82 ± 0.10, $P < 0.05$; DMThal: AUC = 0.87 ± 0.092; $P = 0.011$). D-G, The BLA, MEA, and DMThal are able to differentiate between the eFSE-NoEpi and eFSE-Epi groups (D, E, F), whereas the entorhinal cortex does not (G). One-way analysis of variance with Bonferroni multiple comparison test (BLA: control vs eFSE-NoEpi, $P = 0.89$; control vs eFSE-NoEpi, $P < 0.001$; eFSE-NoEpi vs eFSE-Epi, $P < 0.001$; MeA: control vs eFSE-NoEpi, $P > 0.99$; control vs eFSE-NoEpi, $P < 0.001$; eFSE-NoEpi vs eFSE-Epi, $P = 0.001$; DMThal: control vs eFSE-NoEpi, $P < 0.001$; control vs eFSE-NoEpi, $P < 0.001$; eFSE-NoEpi vs eFSE-Epi, $P < 0.05$; entorhinal cortex: control vs eFSE-NoEpi, $P > 0.99$; control vs eFSE-NoEpi, $P = 0.07$; eFSE-NoEpi vs eFSE-Epi, $P = 0.11$), $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ns, not significant

females were used and randomly assigned to both groups (Figure 3).

- Cohort 4: Imaged in vivo at 11.7 T 2, 48, and 96 hours after eFSE (10 controls, 11 eFSE). Both males and females were used and randomly assigned to both groups (Figure 4).
- Cohort 5: Imaged ex vivo at 9.4 T during early adulthood (3.5 ± 0.8 months; 9 controls, 4 eFSE). Both males and females were used and randomly assigned to both groups (Figures 5 and 6).

2.2 | In vivo MRI procedure

For all MRI studies, rats were lightly anesthetized using 1.5% isoflurane in 100% O_2 to minimize motion, and body temperature was maintained at ~37°C with a heated water cushion.

2.3 | 11.7-T in vivo MRI

A single Avance 11.7-T (Bruker Biospin, Billerica, Massachusetts) magnetic resonance scanner (Loma Linda

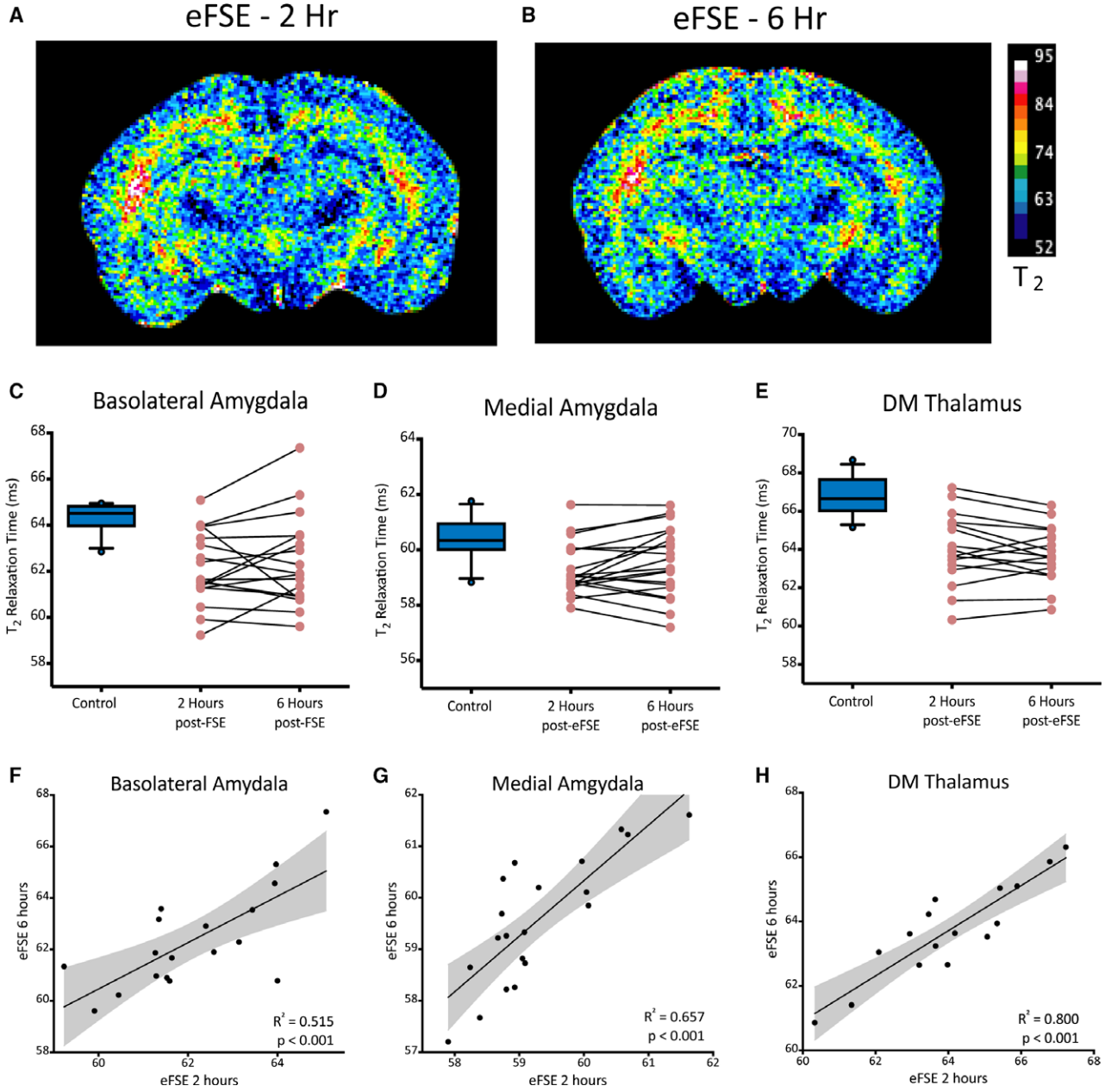

FIGURE 2 Magnetic resonance imaging T_2 values do not change significantly between 2 and 6 hours. A, B, Representative T_2 maps of a rat at 2 and 6 hours after experimental febrile status epilepticus (eFSE). C-E, The majority of rats remain in the same predictive group compared to controls at 2 and 6 hours after eFSE (paired *t* test, basolateral amygdala [BLA]: $P = 0.45$, $t = 0.78$, $df = 17$, correlation $r = 0.72$, $P < 0.001$; medial amygdala [MeA]: $P = 0.11$, $t = 1.691$, $df = 19$, correlation $r = 0.81$, $P < 0.001$; dorsal medial [DM] thalamus: $P = 0.19$, $t = 1.36$, $df = 15$, correlation $r = 0.89$, $P < 0.001$). F-H, Strong correlations between 2- and 6-hour time points for the BLA, MeA, and DM thalamus

University Center for Imaging Research) was used for all 11.7-T T_2 studies for Cohorts 1, 2, and 4. T_2-weighted images were acquired using a two-dimensional multiecho–spin-echo sequence with a Bruker Biospin circular radiofrequency coil and the following parameters: field of view = 2.3 × 2.3 cm, slice thickness = 0.75 mm, repetition time = 4697 milliseconds; echo time (TE) = 10.21-100.1 milliseconds, inter-TE = 10.21 milliseconds, matrix size: 192 × 192, number of averages = 2.

2.4 | 3.0-T in vivo MRI

Cohort 3 rats underwent 3.0-T T_2 imaging on a single Phillips (Best, The Netherlands) Achieva 3.0-T magnetic resonance scanner (University of California, Irvine Neuroscience Imaging Center). T_2-weighted images were acquired using a clinical wrist coil a two-dimensional multiecho–spin-echo sequence with the following parameters: field of view = 2.3 × 2.3 cm, matrix size = 152 × 153,

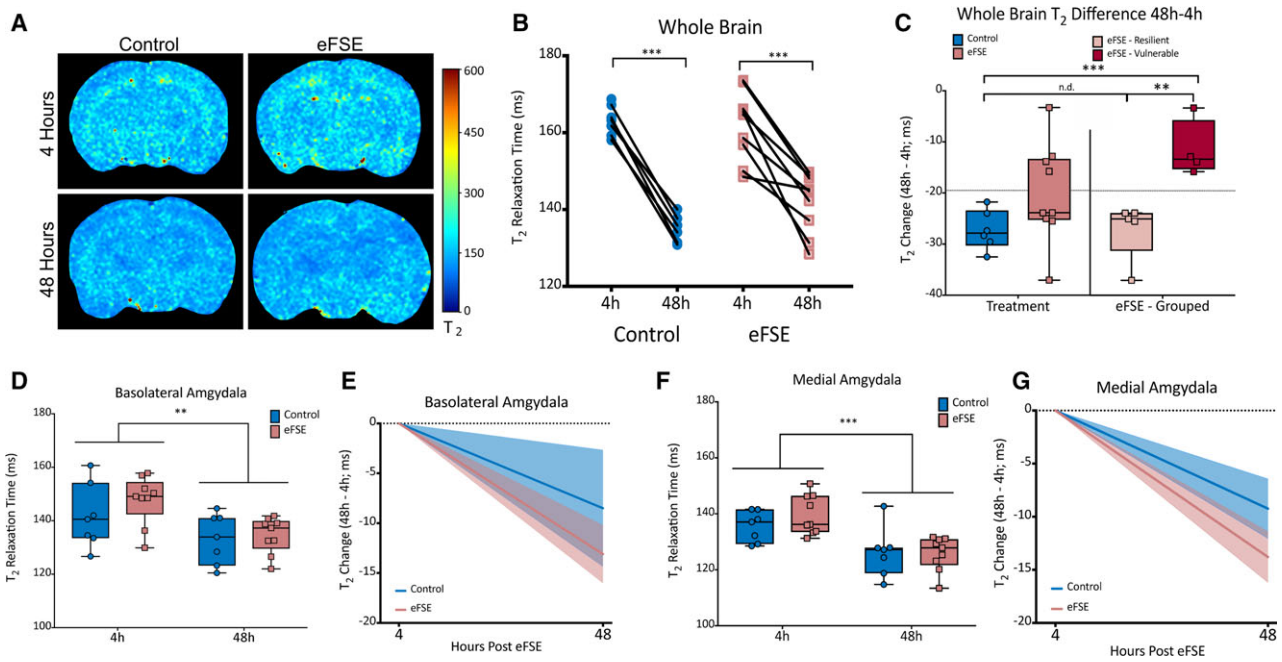

FIGURE 3 Immature rat magnetic resonance imaging 4 and 48 hours after experimental febrile status epilepticus (eFSE) in a human 3.0-T scanner is able to differentiate groups of eFSE rats at a whole brain level but not in individual regions. A, Representative 3.0-T T_2 maps at 4 and 48 hours after eFSE. B, Whole brain 3.0-T T_2 values revealed consistent reductions in all control rats between 4 and 48 hours, but eFSE rats exhibit increased variability in their trajectories (paired *t* test, control: $P < 0.001$, $t = 17.23$, $df = 5$; eFSE: $P = 0.003$, $t = 6.177$, $df = 8$). C, Delta T_2 (48 vs 4 hours) did not differentiate between the eFSE and control groups, but two clear groups within the eFSE emerge (*t* test, control vs eFSE: $P = 0.12$). By separating the eFSE animals into those with a similar trajectory as controls, and those with a smaller T_2 decrease, the two groups were statistically different from each other and eFSE-resilient was different from controls (dividing line at control + 2 SD, $\Delta \geq 19.5$; one-way analysis of variance [ANOVA], eFSE-vulnerable vs eFSE-resilient: $P < 0.01$; eFSE-vulnerable vs control: $P < 0.001$; eFSE-resilient vs control: $P > 0.99$). D-G, In vivo imaging of immature rats in a human 3-T scanner did not reveal regional differences between groups (two-way ANOVA, no significant interaction between treatment group and time, significant effect of time; basolateral amygdala: $F_{1,\ 14} = 12.62$, $P < 0.01$; medial amygdala: $F_{1,\ 14} = 39.54$, $P < 0.0001$). $^{**}P < 0.01$, $^{***}P < 0.001$, n.d., no difference

slice thickness = 1.0 m, slice interval = 0.1 mm, repetition time = 2000 milliseconds, TE = 17.20-51.6 milliseconds, inter-TE = 17.20 milliseconds, number of averages = 2.

2.5 | In vivo MRI analysis

Absolute T_2 relaxation times (in milliseconds) were calculated by log transform followed by linear least-squares fit on a pixel-by-pixel basis, and T_2 maps were generated using in-house software (MATLAB; MathWorks, Natick, Massachusetts).

For all in vivo studies, regions were delineated manually without knowledge of treatment group using ImageJ software (versions 1.25-2.0.0). The regions of interest (ROIs) were drawn with a pseudocolored lookup table (16 colors) to highlight borders between adjoining regions. All ROIs (shown in Figure S1) were delineated by the same investigator (M.M.C.), with a high level of intrarater reliability (intraclass correlation coefficient: BLA = 0.872, medial amygdala [MeA] = 0.832, dorsal medial thalamus [DMThal] = 0.978, whole brain = 0.992). The whole brain ROI was the entire brain on two consecutive slices, with the anterior slice aligning with the anterior BLA. MRI signal changes are often unilateral and always asymmetrical in children after FSE,[11,23] which is consistent with our previous findings.[8,14] Thus, we performed separate measurements and analysis of each side for all bilateral structures. Based on the results of the Choy et al studies,[8] we a priori chose to analyze data only from the side with the lower T_2. However, to ensure that there are no differences across time points, we present bilateral data in Figure 2. For Cohort 1, the following ROIs were manually drawn: dorsal hippocampus, ventral hippocampus, entorhinal cortex, piriform cortex, cerebellum, MeA, BLA, medial thalamus, and corpus callosum. For the remaining in vivo cohorts, only BLA, MeA, and DMThal were delineated.

2.6 | Ex vivo 9.4-T MRI acquisition and analysis

Cohort 5 rats (~3 months old) were anesthetized with a lethal dose of pentobarbital and perfused with 4%

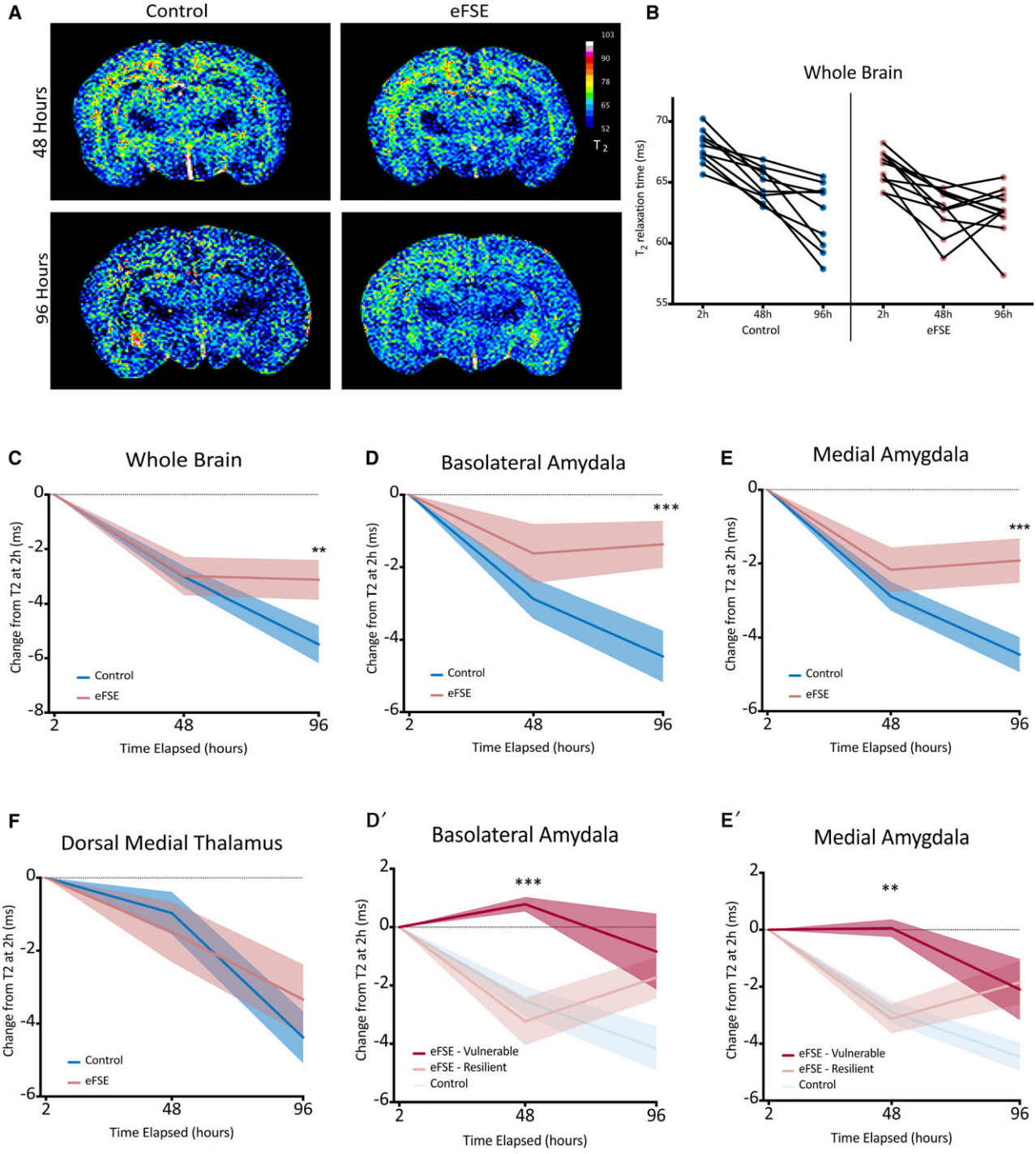

FIGURE 4 The 48-hour 11.7-T magnetic resonance imaging trajectories persist through 96 hours. A, Representative 11.7-T T_2 maps for controls and 48 and 96 hours after experimental febrile status epilepticus (eFSE). B, C, Individual 11.7-T whole brain T_2 values of eFSE and control rats revealed a decreased rate of change at 96 hours following eFSE. D, E, F, There was a significant difference in the trajectories from baseline for the basolateral amygdala (BLA) and medial amygdala (MeA) between control rats and rats that underwent eFSE, but no differences in the dorsal medial thalamus. D′, E′, For the BLA and MeA, separating the eFSE rats into two groups, those that followed a similar trajectory as control rats from 4 to 48 hours (eFSE-resilient; n = 6), and those with a small or no decrease (eFSE-vulnerable; Δ48-4 hours > control + 2 SD; n = 4), revealed two distinct trajectories (BLA, repeated measures [RM] two-way analysis of variance [ANOVA], Šidák multiple comparison test: significant interaction, $F_{4,\ 34} = 5.46$, $P < 0.01$; 48 hours control vs eFSE-vulnerable, $P < 0.01$; eFSE-vulnerable vs eFSE-resilient, $P < 0.001$; 96 hours control vs eFSE-vulnerable, $P < 0.01$; control vs eFSE-resilient, $P < 0.05$; MeA, RM two-way ANOVA, Šidák multiple comparison test: significant interaction, $F_{4,\ 34} = 6.60$, $P < 0.001$; 48 hours control vs eFSE-vulnerable, $P < 0.01$; eFSE-vulnerable vs eFSE-resilient, $P < 0.01$; 96 hours control vs eFSE-vulnerable, $P < 0.05$; control vs eFSE-resilient, $P < 0.001$). $^{**}P < 0.01$, $^{***}P < 0.001$

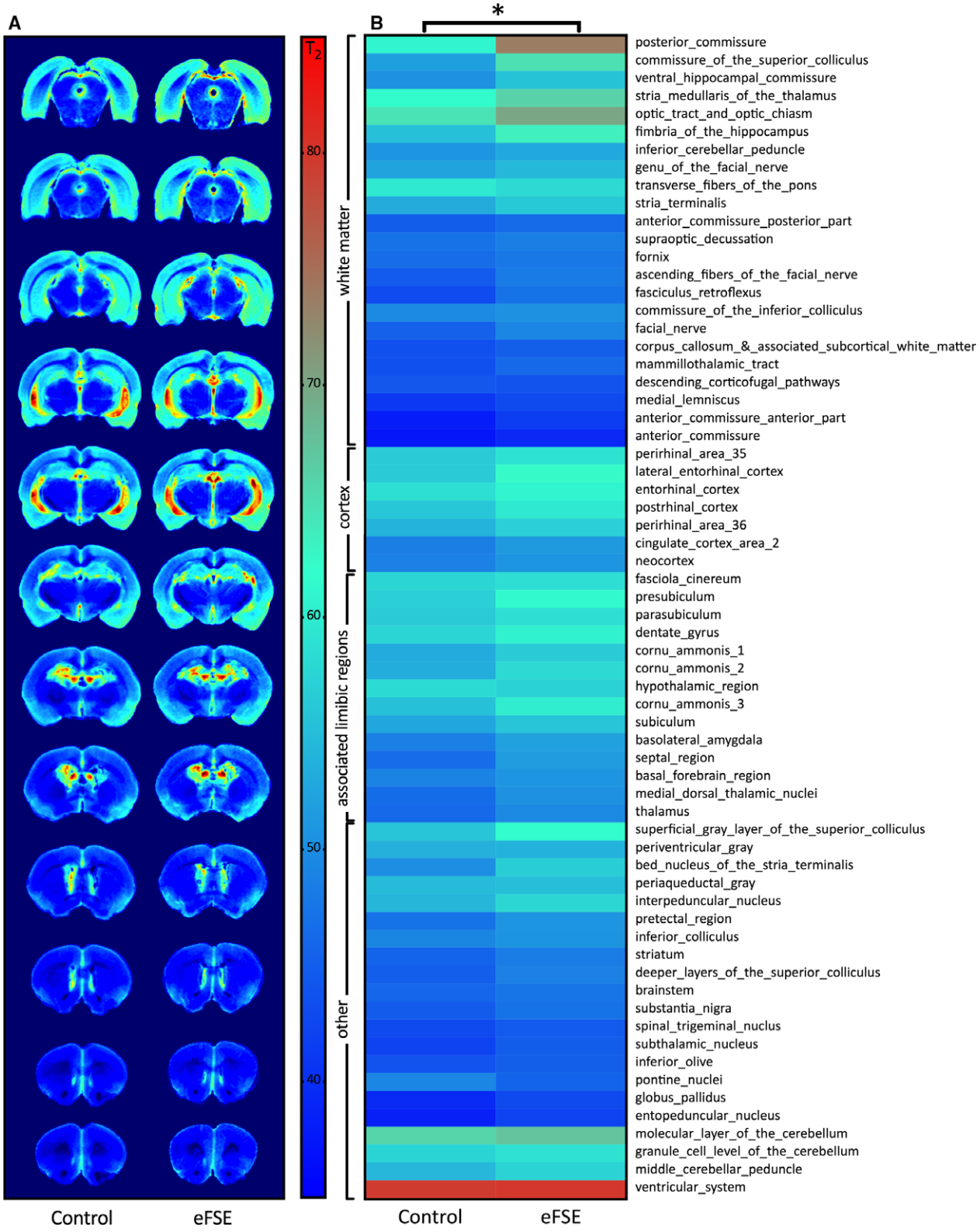

FIGURE 5 Magnetic resonance imaging of adult rats (ex vivo) that underwent experimental febrile status epilepticus (eFSE) revealed increased T_2 throughout the whole brain. A, Group averaged images of control and eFSE rats demonstrate the globally elevated T_2 values in the eFSE group. (B) Heat map of brain regions compartmentalized by region type (white matter, cortex, limbic associated regions, other), revealing increased in T_2 relaxation times in FSE animals (n = 4) relative to controls (n = 9), particularly in limbic and cortical regions. A significant difference between the eFSE and control groups was observed when comparing all brain regions (two-way ANOVA, effect of eFSE, $F_{1,\ 11} = 7.17$, $P < 0.05$). *$P < 0.001$

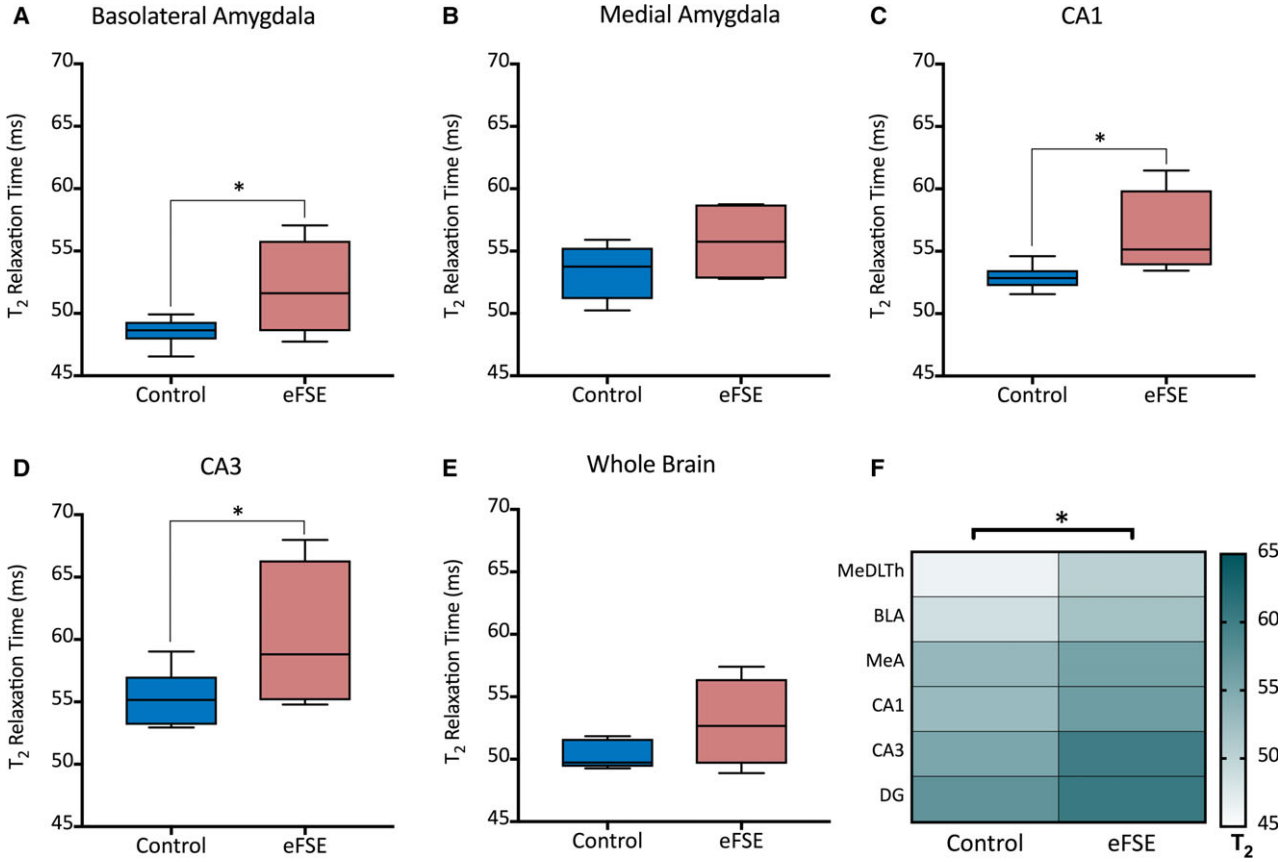

FIGURE 6 A-E, Limbic regions revealed increased T_2 in adult animals that underwent experimental febrile status epilepticus (eFSE) in early life. F, There is a significant T_2 increase in the basolateral amygdala (BLA), cornu ammonis of the hippocampus 1 (CA1), and CA3, with an increase trend across all limbic regions as shown in the heatmap (individual *t* test, BLA: $P < 0.05$, *t* ratio = 2.63, $df = 11$; medial amygdala [MeA]: $P = 0.13$, *t* ratio = 1.64, $df = 11$; CA1: $P < 0.05$, *t* ratio = 2.80, $df = 11$; CA3: $P < 0.05$, *t* ratio = 2.25, $df = 11$; whole brain: $P = 0.057$, *t* ratio = 2.12, $df = 11$; group comparison: two-way analysis of variance, effect of eFSE, $F_{1, 11} = 5.87$, $P < 0.05$). *$P < 0.05$. MeDLTh, medial dorsal thalamic nuclei

paraformaldehyde, and brains were removed and postfixed for 24 hours in paraformaldehyde. Postfixed brains submerged in Fluorinert FC-770 (Sigma-Aldrich, Saint Louis, Missouri) and T_2-weighted images were acquired on a 9.4-T Bruker Biospin MRI scanner (Paravision 5.1; Experimental Imaging Centre, University of Calgary, Calgary, Canada). The 10-echo T_2-weighted images had the following parameters: fifty 0.5-mm slices, 1.92 cm^2 field of view, 256 × 256 matrix, repetition time = 6500 milliseconds, TE = 10 milliseconds, number of averages = 4, resulting in a total scan time of 15 minutes. Quantitative T_2 maps were processed on JIM software (Xinapse Systems, West Bergholt, Essex, UK). The Waxholm MRI atlas[24] was registered to each individual's structural image, and the Waxholm label atlas (separated by hemisphere in JIM) was transformed to this resulting image using Advanced Normalization Tools.[25] Native-space T_2 values were extracted using the transformed Waxholm labels, and, consistent with acute measures, only the unilateral lower T_2 values are presented. To create mean images (Figure 5A), the T_2 maps were registered to a representative control rat's space using FMRIB Software Library's linear registration tool, FLIRT, and averaged by group.

2.7 | EEG electrode implantation and long-term video-EEG recordings and analysis

At ~P40, cohort 1 rats had bipolar electrodes (Plastics One, Roanoke, Virginia) implanted bilaterally in the hippocampus (anteroposterior, 3.3; lateral, 2.3; ventral, −2.8 mm to bregma), a cortical electrode was placed over the parietal cortex (anteroposterior, 2; lateral, −2 mm), and a ground electrode was placed over the cerebellum. EEG recordings were synchronized to video and conducted in freely moving rats beginning 5 days after electrode implantation for up to 10 months, progressively increasing monitoring time to optimize seizure detection. Recordings increased from 112 hours in the first month (15.6% of the time) to

206 hours in the final month (3- to 5-day segments; 28.6% of the month). Over 37 000 hours of video EEG were acquired, including 595 ± 57 hours per control rat and 1319 ± 38 hours per eFSE rat.

EEGs were scanned visually for seizures by two experienced investigators who were blinded to group identity and then reanalyzed using seizure-detection software (LabChart v7.3; ADInstruments, Bella Vista, Australia), and concurrent video recordings were analyzed for behavioral manifestations of seizures. These included sudden cessation of activity, facial automatisms, head bobbing, prolonged immobility with staring, alternating or bilateral clonus, rearing, and falling.[26] Only events with both EEG and behavioral changes that lasted >20 seconds were classified as seizures, and rats with at least one seizure were categorized as epileptic. The analysis and results defining the groups are presented in detail in Choy et al.[8]

2.8 | Statistics

Statistics and graphs were completed on Prism (v7.0; GraphPad, San Diego, California), except the intraclass correlation coefficients, which were calculated using the VassarStat online calculator.[27] Data are presented as box and whisker plots, with bars representing the minimum and maximum, with significance set at $P < 0.05$. Outliers were excluded prior to analysis using the ROUT test ($Q = 1\%$).[28] A list of outliers removed is presented in Table S1. To determine whether MRI performed better than chance at predicting epilepsy after eFSE, regional MRI data from eFSE rats underwent receiver operating characteristic (ROC) analysis. Paired *t* test was used to determine significant change in the whole brain T_2 relaxation time over 48 hours, as well as between 2 and 6 hours for all ROIs, as well as to measure group effects of the adult T_2 images. MRI T_2 relaxation differences in individual regions were compared using one-way repeated measures analysis of variance (ANOVA) with Bonferroni correction. To compare the group effects across time, repeated measures (RM) two-way ANOVA was used with Šidák multiple comparison test.

3 | RESULTS

3.1 | Dynamic changes over 48 hours in 11.7-T T_2 relaxation times are a robust predictor of epileptogenesis

In Cohort 1, six of 19 rats developed spontaneous seizures months after eFSE (31.6%),[8] outlined in Table 1. As reported previously, T_2 signal 2 hours after eFSE in the BLA and other limbic regions was a good predictor of subsequent epileptogenesis. However, the prediction was incomplete, with the BLA predicting epilepsy at 83.3% sensitivity and 76.9% specificity (Figure 1C, inset).

TABLE 1 Description of seizures in Cohort 1

Rat ID	Seizures, n	Mean seizure duration, s	Mean Racine scale	Seizures/24 h recording
6	4	64.50	2.00	0.11
7	3	153.30	1.66	0.04
8	8	99.20	2.13	0.16
9	1	75.00	1.00	0.02
17	4	87.20	2.00	0.05
26	3	84.00	2.33	0.06

We extended these early studies by analyzing the longitudinal change in MRI signals 2 and 48 hours after eFSE, computing the difference in T_2 relaxation times for each region of interest (Figure 1A). Consistent with normal myelination and maturation,[29,30] we found a reduction in T_2 relaxation time in the whole brain of both control rats and the eFSE rats that did not develop epilepsy (eFSE-NoEpi) (paired *t* test, controls: $t = 7.94$, $df = 12$, $P < 0.001$; eFSE-NoEpi: $t = 5.54$, $df = 11$, $P < 0.001$). Surprisingly, the T_2 relaxation decrease was blunted across the whole brain of the eFSE rats that developed epilepsy later in life (eFSE-Epi; paired *t* test, $t = 2.02$, $df = 5$, $P = 0.10$; Figure 1B). These findings suggest that a disruption in the developmental reduction of T_2 relaxation times throughout the brain may be predictive of epileptogenesis.

In our prior work, the BLA was the strongest predictor of epileptogenesis.[8] Here, we found that the longitudinal difference in T_2 relaxation times from 2 to 48 hours following eFSE was a better predictor of epilepsy than the BLA at 2 hours alone. The trajectory of T_2 values in the BLA of eFSE-Epi rats increased over the 48 hours following eFSE, whereas T_2 relaxation times for both the controls and eFSE-NoEpi rats decreased (one-way ANOVA: eFSE-Epi vs controls, $P < 0.001$; eFSE-Epi vs eFSE-NoEpi, $P < 0.001$; Figure 1D).

Examining the potential value of other limbic structures, we discovered that the T_2 difference in MeA was an even a better predictor of epilepsy than BLA (Figure 1E). In MeA, like the BLA, the difference in T_2 relaxation times (48 vs 2 hours) increased over time in eFSE-Epi compared to either controls or eFSE-NoEpi rats (one-way ANOVA: eFSE-Epi vs controls, $P < 0.001$; eFSE-Epi vs eFSE-NoEpi, $P = 0.001$). The dynamic changes of the DMThal also identified differences following eFSE (Figure 1F; control vs eFSE-NoEpi, $P < 0.001$; control vs eFSE-Epi, $P < 0.001$; eFSE-NoEpi vs eFSE-Epi, $P = 0.02$).

The findings were selective, as these predictive changes were not observed in other limbic regions such as entorhinal cortex (Figure 1G; control vs eFSE-NoEpi, $P > 0.99$; control vs eFSE-Epi, $P = 0.07$; eFSE-NoEpi vs eFSE-Epi, $P = 0.11$). The same pattern of results was found when comparing the T_2 values using a two-way ANOVA; the BLA, MeA, and DMThal had a significant interaction between time and outcome. Notably, no interaction was found in the entorhinal cortex (Figure S2). The predictive power of the T_2 trajectory persisted when the individual T_2 values were normalized to the whole brain T_2 values, establishing that the results were not solely due to whole brain reduction of T_2 values. No significant correlation was found when comparing the T_2 change within the whole brain, BLA, MeA, or DMThal with the seizure burden of the individual animals.

The predictive efficacy of the T_2 difference in the BLA, MeA, and DMThal as a marker of epileptogenesis was confirmed by the use of an independent unbiased measure, the ROC curve analysis. ROC curve analysis is an unbiased measure of how successful a test is at predicting the outcomes of a group of subjects. We used it to compare the predictions of epileptogenesis versus the actual outcomes of the eFSE-NoEpi group and eFSE-Epi group. The curve for a test that has 100% sensitivity and 100% specificity will follow the top left corner and will have an area under the curve of 1.00, whereas a test that performs as chance will be at 45° across the graph with an area under the curve of 0.50 (represented by the gray line in Figure 1C). Using the ROC, the predictive value of the T_2 differences was very robust (Figure 1C; area under the curve, BLA: 0.99 ± 0.02, $P < 0.001$; MeA: 1.00 ± 0.00, $P < 0.001$; DMThal: 0.87 ± 0.09, $P = 0.01$).

3.2 | Longevity of MRI changes of T_2 relaxation times after eFSE

A challenge to the clinical translation of our original findings in Choy et al[8] was the potential need to image children sustaining FSE within 2-4 hours after the initial insult. Therefore, we imaged a cohort of rats at both 2 and 6 hours after eFSE (Figure 2A and 2B). There was a strong correlation within each rat and no effect of time between 2 and 6 hours at a group level for T_2 values in the BLA, MeA, DMThal, and whole brain (Figure 2C-H; BLA, paired t test: $P = 0.45$, $t = 0.78$, $df = 17$, correlation $r = 0.72$, $P < 0.001$; MeA, paired t test: $P = 0.11$, $t = 1.691$, $df = 19$, correlation $r = 0.81$, $P < 0.001$; DMThal, t test: $P = 0.99$, $t = 1.36$, $df = 15$, correlation $r = 0.89$, $P < 0.001$; whole brain [Figure S3], t test: $P = 0.61$, $t = 0.53$, $df = 9$, correlation $r = 0.75$, $P = 0.01$). These data demonstrate the stability of the epilepsy-predicting signal between 2 and 6 hours and indicate that imaging children at 6 hours after FSE could enable prediction of epilepsy.

3.3 | Detection of T_2 changes after eFSE is feasible in a clinically relevant low-field scanner

We imaged rat pups at 4 and 48 hours after eFSE on a 3.0-T human scanner, the current standard for clinical MRIs (Figure 3A). The whole brain T_2 trajectories recapitulated those observed from a high-field scanner (11.7 T); controls had a significant and consistent reduction across the 2 days (Figure 3B; control, paired t test: $P < 0.001$, $t = 17.23$, $df = 5$). The eFSE group also had a significant decrease, but with increased variance ($P < 0.001$, $t = 6.177$, $df = 8$). When analyzing whole brain T_2 changes (48 vs 4 hours), two distinct eFSE groups became apparent, which we termed eFSE-vulnerable and eFSE-resilient (Figure 3B). The eFSE-resilient rats fell within 2 SD of the controls, demonstrating the expected reduction of T_2 relaxation time similar to the controls (one-way ANOVA, $P > 0.99$). The eFSE-vulnerable rats were characterized by a minimal reduction of T_2 and were significantly different from both controls and eFSE-resilient groups (one-way ANOVA, eFSE-vulnerable vs eFSE-resilient: $P < 0.01$; eFSE-vulnerable vs control: $P < 0.001$). There was no significant difference either between the T_2 relaxation times (Figure 3D and 3F) or in the change over time (Figures 3E, 3G, and S4) in the BLA or MeA, likely a result of their small volume and the lower resolution inherent in imaging at 3 T (two-way ANOVA, no significant interaction between treatment group and time; significant effect of time for both BLA and MeA, BLA: $F_{1,14} = 12.62$, $P < 0.01$; MeA: $F_{1,14} = 39.54$, $P < 0.0001$).

3.4 | MRI T_2 trajectory from days to months after eFSE

To extend our understanding of the acute and chronic effects of eFSE on T_2 relaxation time trajectories, we imaged eFSE rats at additional acute time points and in adulthood. Delineating acute trajectories, (4, 48, and 96 hours after eFSE; Figure 4A) we found differing developmental patterns for control and eFSE rats across the whole brain and within specific limbic regions. Control rats had consistent reductions in individual T_2 whole brain trajectories across 96 hours, whereas the eFSE group was more variable (Figure 4B). At a group level, there was a significant interaction for the whole brain between the effect of eFSE and imaging time point (RM two-way ANOVA, significant interaction, $F_{2,36} = 3.84$, $P < 0.05$), specifically at 96 hours (Šidák multiple comparison, $P < 0.01$). Developmental patterns of T_2 relaxation times within the BLA and MeA resembled those of whole brain. Notably, patterns within DMThal differed from those of amygdala nuclei; the age-dependent reduction of T_2 in controls appeared delayed, commencing mainly after 48 hours,

and the eFSE-induced inflection of the developmental trajectory, found in BLA, MeA, and whole brain, was not observed (Figures 4D-F and S5A-D; BLA: significant interaction, $F_{2,\ 36} = 5.05$, $P < 0.05$, 96 hours Šidák $P < 0.001$; MeA: significant interaction, $F_{2,\ 36} = 5.40$, $P < 0.01$, 96 hours Šidák $P < 0.001$; DMThal, no interaction, significant effect of time, $F_{2,\ 36} = 22.5$, $P < 0.001$).

The large variance within the eFSE group, especially in T_2 for BLA and MeA at 48 hours following eFSE, prompted us to examine whether the eFSE group was comprised of eFSE-resilient and eFSE-vulnerable subsets. We separated the rats that were within 2 SD of controls at 48 hours following eFSE (eFSE-resilient; BLA/MeA, n = 6) from those outside of that range (eFSE-vulnerable, BLA/MeA, n = 4). For both BLA and MeA, this stratification resulted in two distinctive trajectories over the 48 hours between the eFSE-vulnerable and eFSE-resilient groups, which converged at 96 hours (Figures 4D′, 4E′, S5B′, and S5C′). For both BLA and MeA, interaction was significant between time and treatment (RM two-way ANOVA, Šidák significant interaction, BLA: $F_{4,\ 34} = 5.46$, $P < 0.01$; 48 hours control vs eFSE-vulnerable, $P < 0.01$; eFSE-vulnerable vs eFSE-resilient, $P < 0.001$; 96 hours control vs eFSE-vulnerable, $P < 0.01$; control vs eFSE-resilient, $P < 0.05$; MeA: $F_{4,\ 34} = 6.60$, $P < 0.001$; 48 hours control vs eFSE-vulnerable, $P < 0.01$; eFSE-vulnerable vs eFSE-resilient, $P < 0.01$; 96 hours control vs eFSE-vulnerable, $P < 0.05$; control vs eFSE-resilient, $P < 0.001$). Whereas the basis for these apparent subgroups is unclear, it may suggest that the eFSE-vulnerable group demonstrated an immediate (0-48 hours) delay in the developmental trajectory of T_2 relaxation, whereas a disruption of this T_2 developmental trajectory emerged later in the eFSE-resilient group.

The long-term temporal profile of eFSE-induced changes in T_2 was examined in adults on a 9.4-T scanner (age = 3.5 ± 0.8 months). We found a brain-wide T_2 increase in adult eFSE rats compared to controls, as apparent from the group averaged T_2 maps (Figure 5A). When whole brain regions were parsed, there was a significant effect of eFSE (Figure 5B, two-way ANOVA, effect of eFSE, $F_{1,\ 11} = 7.17$, $P < 0.05$). T_2 increases were observed in eFSE rats in the limbic regions that predicted epileptogenesis (Figure 6A-E), particularly the BLA (t test, $P < 0.05$, $t = 2.63$, $df = 11$). Interestingly, similar findings were noted for the CA1 and CA3 of the hippocampus, regions that had not shown acute changes (CA1: $P < 0.05$, $t = 2.80$, $df = 11$; CA3: $P < 0.05$, $t = 2.25$, $df = 11$). In contrast, T_2 relaxation times in the MeA, medial dorsal thalamic nuclei (MeDLThal), and dentate gyrus were not significantly different between eFSE and control groups. A group comparison of the aggregate BLA, MeA, hippocampal regions, and MeDLThal between eFSE and control rats did provide a robust measure of the consequences of eFSE, revealing a significant effect of eFSE on T_2 values (Figure 6F; two-way ANOVA, effect of eFSE: $F_{1,\ 11} = 5.87$, $P < 0.05$).

4 | DISCUSSION

Here, we provide novel information regarding the trajectories of T_2 imaging–related brain changes that occur in both the short and long term following eFSE. The principal discoveries are: (1) acute longitudinal MRI trajectories following eFSE enhance prediction of epileptogenesis over a single MRI scan; (2) MRI changes following eFSE persist for at least 6 hours; (3) T_2 changes following eFSE can also be measured on clinical MRI scanners; and (4) increased T_2, emblematic of post-FSE changes in children, can be observed in eFSE-experiencing rats months after the insult.

4.1 | Progressive changes in T_2 following eFSE offer improved prediction of epileptogenesis

Our work previously discovered high-field T_2 signal changes as a predictive marker of epileptogenesis immediately (2 hours) following a single episode of eFSE.[8] This was a critical first step in the ability to predict epilepsy before the first spontaneous seizure occurred. Although the prediction was robust, the positive predictive value was imperfect; one-third of rats that were predicted to become epileptic did not have a spontaneous seizure. By analyzing the change in T_2 rather than at a single time point, our new data demonstrate that the repeated longitudinal T_2 changes (48 vs 2 hours) increased prediction of epileptogenesis, increasing the positive predictive value to 100% and 85.7% in the MeA and BLA, respectively. Additionally, this work revealed dynamic MRI differences at multiple time points (48 and 96 hours), reflecting the fluid process of epileptogenesis. We believe that the improved predictive value is based on the ability of serial MRI to differentiate how the temporal profile of T_2 relaxation values of each individual rat either reflects that normal developmental decrease in T_2 relaxation or differs from it, and that it is a lack of developmental T_2 reduction that is the marker of early processes of epileptogenesis. Further studies are required to uncover the biological mechanisms that underlie the relationship between "normal" T_2 decreases and the flattened trajectory of rats in the early stages of epileptogenesis.

4.2 | Reconciliation of early T_2 reduction and chronic T_2 increases following eFSE

There has been a significant body of work that has found T_2 increases in the brain (particularly hippocampus)

following FSE in both humans and rodents.[10-16] Because of this, the results of Choy et al,[8] which revealed a decrease in T_2 after FSE 2 hours after eFSE and no changes at 48 hours, were unexpected, although there were two important differences between it and previous work. First, the imaging was completed much earlier after eFSE. Second, the rats were imaged on an 11.7-T scanner, which has a higher magnetic field and is affected by paramagnetic changes (T_2*) that can be measured as decreased T_2 values.[8]

Analyzing the longitudinal change of T_2 values between 2 and 96 hours after eFSE made it clear that a T_2 increase in the days after eFSE was potentially masked by typical development. This developmental reduction in T_2 relaxation times was measured across the entire brain and occurs as a result of normal myelination and maturation.[29,30] The trajectory is more rapid in infant rats than it is in humans; thus, the developmental reduction in infants would not play as important a role clinically. Whereas the underlying cellular mechanisms that lead to FSE-induced increased T_2 are not fully understood, they have been postulated to involved edema, based on volumetric human studies,[10,15] or gliosis, as our previous work has reported an increased number and activation of astrocytes following eFSE.[12,20]

Importantly, the trajectory between 4, 48, and 96 hours after eFSE reconciles the early and late T_2 changes. The current findings demonstrate divergent developmental paths of eFSE rats compared with their control littermates. The same regions that have an early T_2 decrease predictive of epileptogenesis demonstrate a later T_2 increase in adulthood. Interestingly, early imaging of the hippocampus was not predictive of epileptogenesis,[8] but late increases in T_2 are robust in both human and rodent images.[11-13,17,18]

4.3 | Clinical translation of predictive T_2 signal changes

The present study addresses two main hurdles for translating the predictive MRI signal to clinic: timeline and scanner strength. The original time point, 2 hours after FSE, would be difficult to achieve in an emergency department setting, due to access to MRI and time required for consent. Our new finding that the early T_2 signal decrease remains stable for up to 6 hours makes future clinical imaging of children feasible.

Additionally, our initial study was performed on research scanners (4.7-11.7 T), which have higher magnetic fields and smaller coils than the human scanners (1.5-3.0 T) that are typically available. To demonstrate that similar results would likely be found in children, we imaged eFSE rat pups on a 3.0-T human scanner. Using a clinical human wrist coil, we observed measurable differences in the brains of rat pups following eFSE, similar to the findings in the high-field scanner. These differences were measurable at the whole brain level, not in specific limbic regions, but this is not surprising due to resolution differences; the voxel volume at 11.7 T was 0.011 mm^3, but 0.023 mm^3 at 3.0 T. This inherent resolution difference should not play as critical a role when imaging children, as the whole brain of P12 rat (which had measurable differences at 3.0 T) is ~1 cm^3 in volume, roughly the same volume of the amygdala of a 1-year-old infant.[31,32] Thus, it is likely the whole brain differences in the rat pup will translate to even stronger measurements when analyzing individual brain regions in children, mirroring the results found in rats on the animal scanners.

In addition to the noted resolution differences, it is important to remember that T_2 relaxation properties are modulated by field strengths, age at imaging, and acquisition parameters. Human and rodent T_2 values are known to decrease with increasing field strength[33,34] (see Choy et al[8] for discussion). In the rodent brain, it has been suggested that signal-to-noise ratios may actually decrease as field strength increases[34]; thus, imaging at the clinically relevant 3 T as we did in this study may enhance our findings at this field strength. In addition, the influence of T_2 values by field strength is similar in white and gray matter even if acquisition parameters differ.[34] Of course, the use of high signal-to-noise radiofrequency coils and purpose-built coils would enhance the acquisition of T_2 signals in neonates and adults.

Overall, this study clearly demonstrates the value of quantitative T_2 MRI as a marker for the alterations in the brain following eFSE that occur at the onset of epileptogenesis. Although long-term EEG recording was only completed in one group of rats following eFSE, the strength of the longitudinal MRI results in the EEG cohort allows us to predict the epileptogenic group utilizing short-term MRI changes in the other cohorts. Moving forward, it will be important to both confirm these findings with a long-term EEG cohort and to study the mechanisms underlying the MRI signal changes. This continued research will inform us how epilepsy develops, help us understand how we can prevent it, and move us toward translating these findings to the clinic.

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DISCLOSURE

The authors have no conflicts of interest to report. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

STATEMENT OF ETHICS

The ARRIVE guidelines and Basel Declarations were adhered to, and efforts were taken in experimental designs to allow for replacement, reduction, and refinement of the animals that were used and to minimize the pain and suffering of those that were included.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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