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RESEARCH ARTICLE

Epilepsy phenotype and its reproducibility after lateral fluid percussion-induced traumatic brain injury in rats: Multicenter EpiBioS4Rx study project 1

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Abstract

Objective: This study was undertaken to assess reproducibility of the epilepsy outcome and phenotype in a lateral fluid percussion model of posttraumatic epilepsy (PTE) across three study sites.

Methods: A total of 525 adult male Sprague Dawley rats were randomized to lateral fluid percussion-induced brain injury (FPI) or sham operation. Of these, 264 were assigned to magnetic resonance imaging (MRI cohort, 43 sham, 221 traumatic brain injury [TBI]) and 261 to electrophysiological follow-up (EEG cohort, 41 sham, 220 TBI). A major effort was made to harmonize the rats, materials, equipment, procedures, and monitoring systems. On the 7th post-TBI month, rats were video-EEG monitored for epilepsy diagnosis.

Results: A total of 245 rats were video-EEG phenotyped for epilepsy on the 7th postinjury month (121 in MRI cohort, 124 in EEG cohort). In the whole cohort ($n = 245$), the prevalence of PTE in rats with TBI was 22%, being 27% in the MRI and 18% in the EEG cohort ($p > .05$). Prevalence of PTE did not differ between the three study sites ($p > .05$). The average seizure frequency was $.317 \pm .725$ seizures/day at University of Eastern Finland (UEF; Finland), $.085 \pm .067$ at Monash University (Monash; Australia), and $.299 \pm .266$ at University of California, Los Angeles (UCLA; USA; $p < .01$ as compared to Monash). The average seizure duration did not differ between UEF (104 ± 48 s), Monash (90 ± 33 s), and UCLA (105 ± 473 s; $p > .05$). Of the 219 seizures, 53% occurred as part of a seizure cluster

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(≥3 seizures/24 h; $p > .05$ between the study sites). Of the 209 seizures, 56% occurred during lights-on period and 44% during lights-off period ($p > .05$ between the study sites).

Significance: The PTE phenotype induced by lateral FPI is reproducible in a multicenter design. Our study supports the feasibility of performing preclinical multicenter trials in PTE to increase statistical power and experimental rigor to produce clinically translatable data to combat epileptogenesis after TBI.

KEYWORDS

harmonization, posttraumatic epilepsy, preclinical, video-EEG monitoring

1 | INTRODUCTION

Traumatic brain injury (TBI) is defined as an alteration in brain function, or other evidence of brain pathology, caused by an external force.[1] Annually, approximately 2.5 million people in both Europe and the USA experience TBI (www.center-tbi.eu; www.cdc.gov/traumaticbraininjury). TBI is a major etiology for structural epilepsy in humans, with posttraumatic epilepsy (PTE) estimated to account for approximately 20% of structural epilepsies and 5% of all epilepsies.[2–4] Despite approximately 20 promising preclinical proof-of-concept antiepileptogenic or biomarker discovery studies in animal models of PTE, none of the interventions or candidate biomarkers has progressed to clinical use.[5]

One of the major obstacles on the path from laboratory to clinic is the low reproducibility of preclinical studies.[6] This often relates to a low sample size, and consequently, an underpowered study.[7] A potential solution to this problem is multisite preclinical randomized controlled trials (pRCTs), but for these to be successful, there is a need for harmonization of data collection with the use of common data elements and standardization of procedures to achieve greater methodological rigor.[8,9] The first centrally coordinated, randomized, and blinded pRCT tested the effect of anti-CD49d antibodies on infarct volume in two stroke models at six European centers.[10] Efforts to conduct preclinical multicenter biomarker and therapy studies in TBI were reported by the Operation Brain Trauma Therapy network.[11] More recently, methods optimization for preclinical stroke intervention studies have been reported by the Italian Stroke Organization (ISO) Basic Science network[12] and the Stroke Preclinical Assessment Network investigators.[13] These projects have demonstrated promising data, supporting the feasibility and benefits of conducting preclinical multicenter trials.

The Epilepsy Bioinformatics Study for Antiepileptogenic Therapy (EpiBioS4Rx) consortium, a National Institute of Neurological Disorders and Stroke-funded Center Without Walls international study, is the first to apply preclinical multicenter design in antiepileptogenesis biomarker identification and therapy development (https://epibios.loni.usc.edu/). Project 1 of the EpiBioS4Rx intends to facilitate the development of antiepileptogenic therapies after TBI through the discovery of preclinical blood, electrophysiologic, and imaging biomarkers of epileptogenesis, using harmonized protocols and statistically powered study designs. EpiBioS4Rx Project 1 was performed at three international sites located in Finland (University of Eastern Finland [UEF], Kuopio), Australia (Monash University [Monash], Melbourne), and the USA (David Geffen School of Medicine at University of California, Los Angeles [UCLA]).

Key Points

- The first large-scale preclinical multicenter study in epilepsy
- Epilepsy phenotype after lateral fluid percussion-induced traumatic brain injury is highly reproducible across different study sites
- Preclinical multicenter studies are feasible

The first objective of EpiBioS4Rx Project 1 was to demonstrate that the three study sites can perform lateral fluid percussion-induced brain injury (FPI)-induced TBI, resulting in PTE with similar prevalence and epilepsy phenotype. At all study sites, a major effort was made to harmonize the materials, equipment, and rats used as well as the procedures and monitoring systems, including blood sampling, video-electroencephalogram (video-EEG), and magnetic resonance imaging (MRI). Our data show that (1) posttraumatic epileptogenesis and epilepsy phenotype induced by FPI-induced TBI in adult male rats is highly reproducible, tolerating some site-specific procedural differences; and (2) application of a multicenter approach is feasible for preclinical studies on epileptogenesis after TBI.

2 | MATERIALS AND METHODS

Figure 1 summarizes the study design that was applied by all three study sites (UEF, Monash, UCLA). Table 1 summarizes the information on animals, feeding, housing, surgery, EEG recording systems, data acquisition, and data analysis at the different study sites. Figure S1 shows the randomization based on preliminary data,[14–16] study flow, and causes of exclusions. Detailed methodologic descriptions, including ethics and statistics, are given in Appendix S1.

3 | RESULTS

Altogether, 245 animals were EEG-phenotyped and included in the final analysis cohort (Figure S1), that is, 121 rats in the MRI cohort and 124 in the EEG

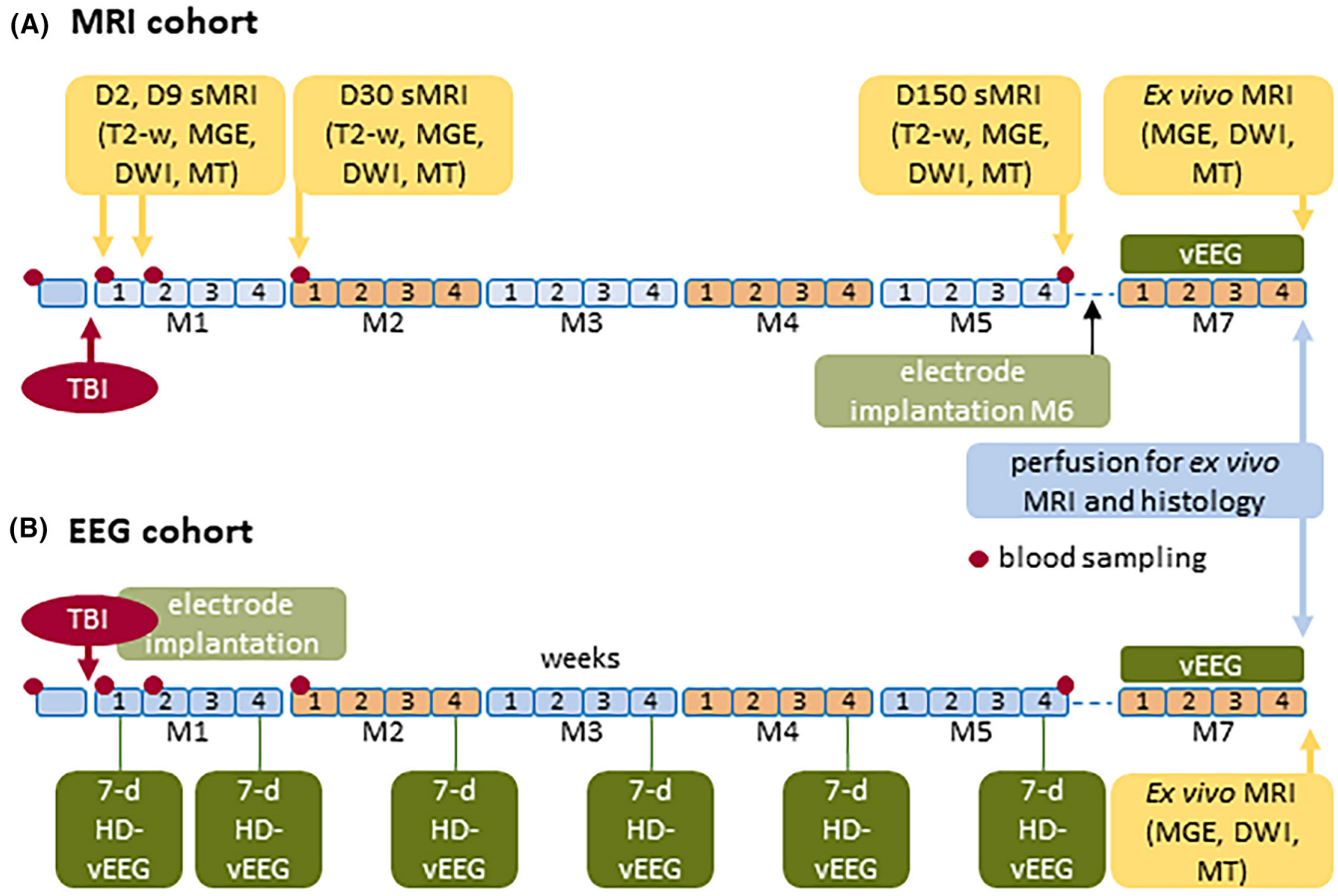


FIGURE 1 Study design. EpiBioS4Rx Project 1 consisted of two separate animal cohorts: the magnetic resonance imaging (MRI) cohort and video-electroencephalography (vEEG) cohort. They were generated and monitored by using the same protocols at the three study sites (University of Eastern Finland [UEF]; Monash University; University of California, Los Angeles [UCLA]). In both cohorts, the rats were randomized either to the sham-operated experimental control or TBI groups. Epileptogenesis was triggered using lateral fluid percussion-induced traumatic brain injury (TBI). During the first follow-up month (M1), rats underwent extensive physiologic and somatomotor monitoring to assess animal well-being and injury severity at different study sites. These included body weight and core temperature (UEF only) at baseline (BL) and on day (D)1, D2, D3, D4, D5, D6, D7, D8, D9, D14, and D30 after TBI (injury day, D0) and composite neuroscore at BL and on D2, D7, D14, D21, and D28. For blood biomarker analysis, tail vein blood sampling was performed in both cohorts at BL and on D2 (48 h), D9, and D30 and 5 months post-TBI. In the MRI cohort, rats were magnetic resonance imaged on D2 (48 h), D9, and D30 and 5 months post-TBI using T2-weighted imaging (T2-w), multiecho gradient echo (MGE) imaging, diffusion-weighted imaging (DWI) for diffusion tensor imaging, and tractography and magnetization transfer imaging (MT). Then, the rats were implanted with epidural and intracerebral electrodes for vEEG. In the EEG cohort, electrodes were implanted right after TBI and rats were recorded during the 1st week, and then monthly with a 1-week-long high-density (HD) EEG for detecting high-frequency oscillations, from right after the TBI or sham operation until the 6th month. During the 7th month, both the MRI and EEG cohorts were continuously (24/7) vEEG monitored for 30 days to detect spontaneous seizures to diagnose posttraumatic epilepsy (no video was recorded at UCLA). Finally, at 7 months after TBI or sham operation, rats were perfused for ex vivo MRI (MGE, DWI and MT modalities), after which the brains were processed for histologic analysis. sMRI, structural MRI.

TABLE 1 Rats, material, and equipment used for model production and video-EEG analysis at the three study sites.

	UEF	Monash	UCLA
Animals and housing			
Number of rats randomized	184	194	147
Strain and species	Sprague Dawley rats	Sprague Dawley rats	Sprague Dawley rats
Vendor (Country)	Envigo Laboratories (the Netherlands)	In-house breeding (Australia)	Charles River (USA)
Sex	Male	Male	Male
Food pellets	2016S (Teklan Diet; Envigo Laboratories)	102108 (Barastoc)	LabDiet 5001 (LabDiet)
Duration of quarantine	7 days	3–7 days	At least 3 days
Lights-on/lights-off cycle	7:00 a.m. lights-on/7:00 p.m. lights-off	7:00 a.m. lights-on/7:00 p.m. lights-off at Monash University and 6:00 a.m. lights-on/6:00 p.m. lights-off at Melbourne University	6:00 a.m. lights-on/6:00 p.m. lights-off
Room temperature, °C	22 ± 1	22 ± 1	20–26
Surgery			
Weight at the time of injury, g (range)	354 ± 18 (315–408)	349 ± 39 (250–440)	336 ± 41 (260–497)
Anesthesia system	Somnosuite #SS6069B (Kent Scientific)	Somnosuite #SS6069B (Kent Scientific)	Matrix VIP 3000 Vaporizer #91305430 (Patterson Veterinary)
Anesthetic	Isoflurane	Isoflurane (5% induction & 2% maintenance)	Isoflurane (VetOne)
Medical oxygen	Not applicable	Mediquip Medical Equipment & Supplies	Not applicable
Trephine	#18004-50 (handheld; Fine Science Tools)	Model 300 (motorized; Dremmel)	#18004-50 (handheld; Fine Science Tools)
Tissue adhesive	3M Vetbond (3M Deutschland)	Octyl cyanoacrylate (Bostik)	3M Vetbond (3M Deutschland)
Dental acrylate	Selectaplus #10009210 or #D10009102 (DeguDent)	AVSCV00500 (Vertex)	SNAP liquid (P16-02-65) and powder (P16-02-60; Pearson Dental)
Fluid percussion device	Model FP 302 (AmScien Instruments)	Model FP 302 (AmScien Instruments)	Model FP 302 (AmScien Instruments)
Analgesia	Buprenorphine (Orion Pharma)	Buprenorphine (Indivior)	Flunixin meglumine (Merck)
Other treatments	None	None	Enrofloxacin (Norbrook)
Additional feeding	Powdered pellets	Milk powder, mixed with powdered pellet and water provided ad libitum, until rats recovered their preinjury weight	Trimethoprim sulfamethoxazole medicated rodent chow
Housing after impact surgery	Single-housed	Single-housed	Single-housed
EEG recording system, materials, and data acquisition			
Head cap	12-channel pedestal (MS12P EM12/20/2.4/SP; Plastics One)	12-channel pedestal (MS12 P EM12/20/2.4/SP; Plastics One)	6-channel pedestal (MS363; Plastics One)
Cable	Flexible shielded cable (M12C-363/2; Plastics One)	Flexible shielded cable (M12C-363/2; Plastics One)	Flexible shielded cable (363/2–363/2; Plastics One)

TABLE 1 (Continued)

	UEF	Monash	UCLA
Commutator	12-channel double-brush (SL12C; (Plastics One)	12-channel double-brush (SL12C, 12-pin swivel; Plastics One)	12-channel double-brush (SL-12C, 12-pin swivel; Plastics One)
Epidural electrodes	E363/20/2.4/Spc (stainless steel; Plastics One)	363/120/2.4 ELEC W/000-120 X 2.4 SCREW or E363/20/SPC ELEC W/3.2MM SCREW SS (Plastics One)	Stainless steel epidural screw (F000CE096, J.I. Morris Co.)
Intracerebral electrodes	EM12/3-2TW/Spc (tungsten, 100 µm) E363T/5-2TW/Spc (tungsten, 50 µm)	E363T/5-2TW ELEC (tungsten, 50 µm)	Bipolar intracerebral tungsten .002″ twisted electrode
Electrode impedance	Initially below 5 KΩ; maintained below 10 KΩ	Below 10 KΩ (measured at 1000 Hz)	Female Socket-Contact E363/0 (Plastics One), below 5 KΩ
Amplifier model	Digital Lynx 16SX (Neuralynx)	Neuvo	Intan RHD2000
Acquisition software	Cheetah v6.3.2	Profusion EEG 5	RHD2000
Sampling rate	5 kHz	2 kHz/channel	2 kHz/channel
Filter settings	FIR high-pass .1 Hz	.01–2030 Hz	.1–1000 Hz
EEG file format	.NCS converted to EDF+	.rda2 converted to EDF+	.RHD converted to EDF+
EEG file duration	24 h	24 h	2 h
EEG file size	~18 GB	~3.8 GB	~1.2 GB
Video system and materials			
Camera	Basler ace acA1300-75gc; Basler	IP Camera (Vivotek)	Not applicable
Video acquisition software	Inhouse software	Profusion 5	Not applicable
Frames per second	30	30	Not applicable
Resolution	1.3 Mpx (1280 px × 1024 px)	2 Mpx	Not applicable
Analysis of video-EEG			
Seizure detection in EEG	Homemade seizure detection algorithm[38] followed by visual validation of positive hits	Either manual seizure detection or Assyst algorithm for automated seizure detection followed by visual validation of positive hits[39]	Manual seizure detection using EDFbrowser software
Assessment of behavioral seizure severity	Racine scoring of video-imaged seizures	Racine scoring of video-imaged seizures	Not applicable

Abbreviation: EEG, electroencephalography; FIR, finite impulse response.

cohort. Of these, 100 rats were generated at UEF, 84 at Monash, and 61 at UCLA. We will first describe the injury characteristics and then the epilepsy characteristics.

3.1 | Impact pressure, postinjury apnea, and duration of righting reflex

Below, we will focus on the site differences. Impact severity and its effects on apnea time and righting reflex in the EEG-phenotyped MRI and EEG cohorts as well as in the animals that did (TBI+) or did not (TBI−) develop epilepsy at different study sites are summarized in Appendix S1 and Table S1.

3.1.1 | Impact pressure

Data were available from 95% (178/187) of the rats with TBI (Table S1). The average impact pressure used was 2.65 ± .37 atm ($n = 178$). The impact pressure varied between the sites ($p < .001$). At UEF, the impact pressure used (2.88 ± .16 atm, $n = 75$) was higher than that at Monash (2.67 ± .37 atm, $n = 59$; adj. $p < .01$) or at UCLA (2.20 ± .20 atm, $n = 44$; adj. $p < .001$). At Monash, the

impact pressure was higher than that at UCLA (adj. $p < .001$).

3.1.2 | Postimpact apnea

Data were available from 100% (187/187) of the rats with TBI (Table S1). The average duration of postimpact apnea was 41 ± 38 s ($n = 187$). The mean postimpact apnea varied between the sites ($p < .001$). At UEF, the apnea duration (28 ± 14 s, $n = 75$) was shorter than that at Monash (67 ± 51 s, $n = 68$; adj. $p < .001$). At UCLA, the apnea duration (22 ± 11 s, $n = 44$) was shorter than that at Monash (adj. $p < .001$) and also shorter than that at UEF (adj. $p < .05$).

3.1.3 | Righting reflex

Data were available from 60% (35/58) of the sham-operated experimental controls and 99% (186/187) of the rats with TBI (Table S1). No data were available from sham-operated animals at UCLA.

The average duration of righting reflex in the TBI group (1057 ± 512 s, $n = 186$) was substantially longer than that in the sham-operated group (187 ± 100 s, $n = 35$; $p < .001$). In the sham-group, the average duration of righting reflex did not differ between UEF and Monash (178 ± 102 s vs. 200 ± 98 s; $p > .05$). In the TBI group, the average duration of righting reflex varied between the sites ($p < .001$). At UEF, the righting time (879 ± 338 s, $n = 74$) was shorter than that at Monash (1347 ± 581 s, $n = 68$; adj. $p < .001$). Also at UCLA, the righting time (910 ± 447 s, $n = 44$) was shorter than that at Monash (adj. $p < .001$). At UEF and UCLA, the righting times did not differ (adj. $p > .05$).

3.2 | Duration of video-EEG recording on the 7th postinjury month

Duration of the index month video-EEG recording at different study sites is summarized in Figure 2C and Table S2. Our objective was to record each animal for 30 days, which was anticipated to give a 99.6% probability of detecting a seizure, if the animal had epilepsy with the expected seizure frequency of .2 seizures/day.[17]

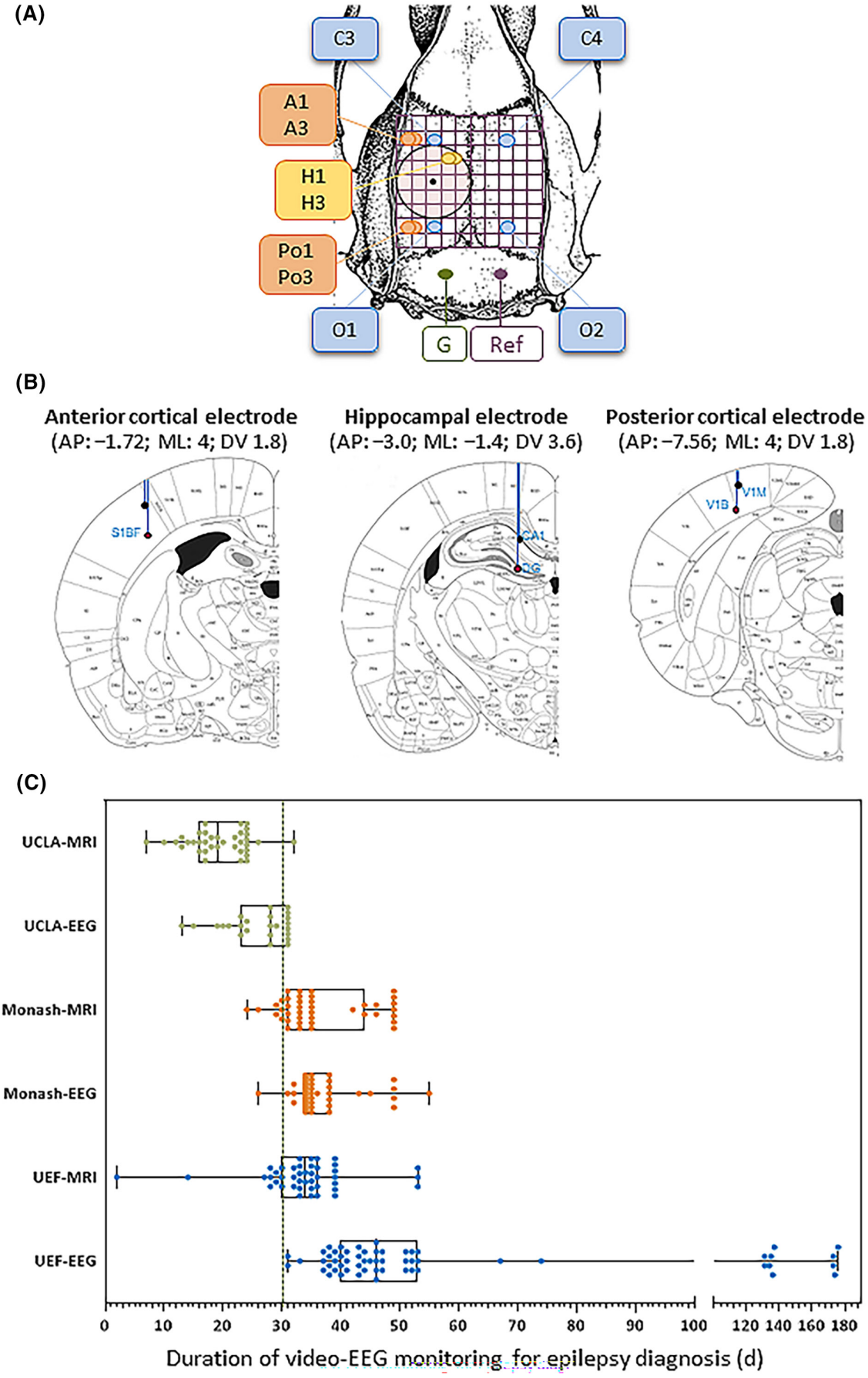
The average duration of the index month (i.e., the 7th month) recording to detect unprovoked (spontaneous) diagnostic seizures for epilepsy was 39 ± 26 days (median = 34 days), ranging from 2 to 176 days. In the UEF MRI cohort, one rat was recorded for 2 days and another for 14 days. The rats had a seizure cluster or status epilepticus (SE) as the "first" seizure, respectively, leading to death. In the UEF EEG cohort, the video-EEG recording times were up to 3 months instead of 30 days, due to the need for additional recordings related to pogo-pin malfunctions. Overall, in 73% (180/245) animals the index month EEG recording lasted for at least 30 days (92% [92/100] at UEF, 94% [79/84] at Monash, 15% [9/61] at UCLA). In the whole animal group as well as at each study site, the duration of index month EEG recordings did not differ between the TBI+ and TBI− groups.

3.3 | Prevalence of epilepsy

An animal was considered to have PTE if it had at least one unprovoked seizure detected during the 7th month video-EEG recording, or at least one handling-related seizure observed by an experienced investigator.[18] Representative examples of electrographic seizures at each study site are shown in Figure S2.

In the whole TBI animal cohort, the prevalence of epilepsy was 22% (41/187), being 27% (24/90) in the MRI and 18% (17/97) in the EEG cohort ($p > .05$; Figure 3). There was no difference in the prevalence of epilepsy between the three study sites ($p > .05$). When each site was assessed separately, the prevalence of epilepsy did not differ between the MRI and EEG cohorts ($p > .05$). At UEF, one rat with video-EEG recorded seizures also had a handling-related seizure. At Monash and UCLA, all seizures detected were electrographic.

FIGURE 2 Epilepsy phenotyping. (A) Electrode placement, showing location of the craniotomy (light pink circle), four epidural recording electrodes (blue; C3, C4, O1, O2), three bipolar intracerebral electrodes (anterior cortical A1 [deeper tip] and A3 [superficial tip]), and hippocampal (H1 and H3), posterior cortical (Po1 and Po3), reference (purple; Ref), and ground (green; G) electrodes in the skull. Grid dimensions = 1 × 1 mm. (B) Coronal plates were adapted from a rat brain atlas,[37] showing the aimed locations of the intracerebral electrode tips (AP, anteroposterior; ML, mediolateral; DV, dorsoventral). (C) Box plots show the duration of video-electroencephalographic (video-EEG) monitoring used to diagnose post-traumatic epilepsy in the EEG and magnetic resonance imaging (MRI) cohorts at different sites. Note that no video monitoring was performed at University of California, Los Angeles (UCLA). The green dashed line shows the aimed duration of the monitoring, that is, 30 days. In 73% (180/245) of the animals, the monitoring lasted for at least 30 days. Details of video-EEG monitoring of different cohorts at different sites are summarized in Table S2. Note that some rats presented status epilepticus as the first seizure and died, resulting in a short monitoring period (University of Eastern Finland [UEF] MRI cohort).



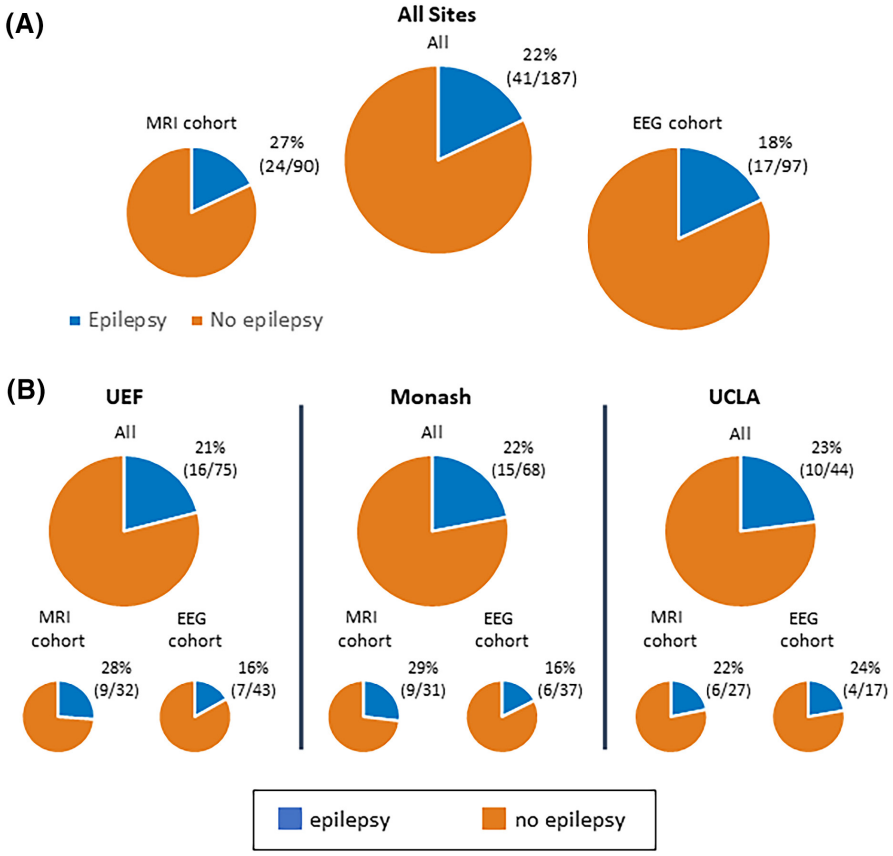


FIGURE 3 Prevalence of epilepsy. Pie charts summarize the prevalence of epilepsy. (A) In the final analysis cohort (all study sites combined), 22% (41/187) of the rats had epilepsy. In the magnetic resonance imaging (MRI) cohort the prevalence was 27% (24/90) and in the electroencephalography (EEG) cohort 18% (17/97). (B) Prevalence of epilepsy at different study sites (University of Eastern Finland [UEF], Monash University [Monash], University of California, Los Angeles [UCLA]). The pie charts at the bottom show the prevalence of epilepsy in the MRI and EEG cohorts at each study site. Prevalence of epilepsy did not differ between the study sites and between the MRI and EEG cohorts.

3.4 | Epilepsy phenotype

A seizure calendar showing the occurrence of all 219 unprovoked electrographic seizures recorded at UEF, Monash, or UCLA is presented in Figure 4. Seizures occurred as isolated events or as part of a seizure cluster or SE.

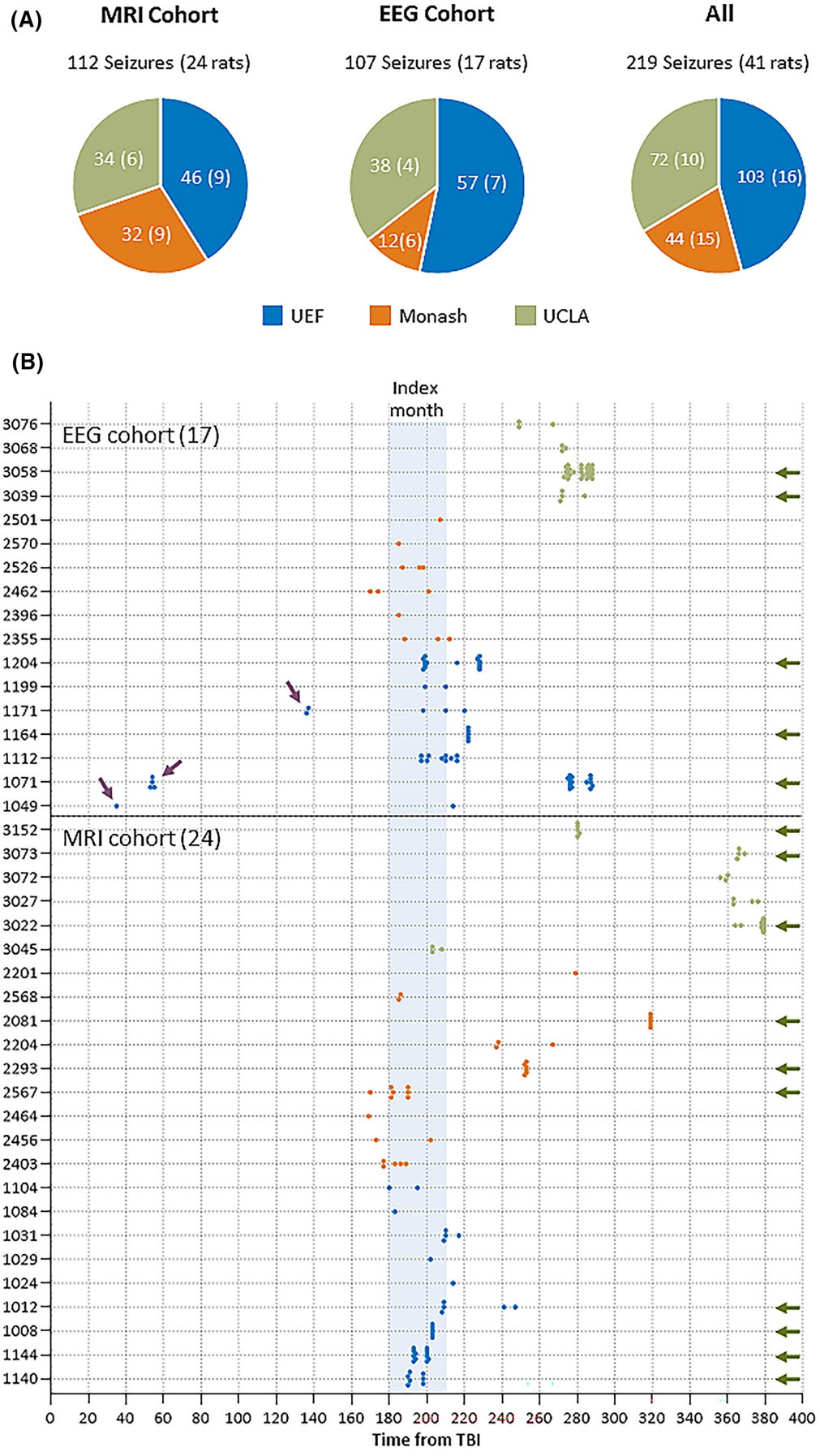
3.4.1 | Seizure frequency

The average seizure frequency (seizures/day) in the whole TBI+ cohort was .228 ± .476 seizures/day (41 rats, median = .106; Figure 5A,B). At UEF, the average seizure frequency was .317 ± .725 seizures/day (16 rats, median = .105), at Monash .085 ± .067 seizures/day (15 rats, median = .065, $n = 15$), and at UCLA .299 ± .266 seizures/day (10 rats, median = .188). Kruskal–Wallis test indicated a difference between the sites ($p < .05$), the seizure frequency being lower at Monash than at UCLA (adj. $p < .01$).

3.4.2 | Seizure duration

The seizure duration was available for 95% (208/219) of the seizures. The average seizure duration was 100 ± 53 s (208 seizures, median = 96 s, range = 13–390 s; Figure 5C,D). The average seizure duration did not differ between UEF (102 seizures, 102 ± 43 s, median = 96 s, range = 13–242 s),

FIGURE 4 Seizure calendar. (A) Number of electrographic seizures in the magnetic resonance imaging (MRI) and electroencephalography (EEG) cohorts and in both cohorts combined at different study sites. Animal numbers are shown in parentheses. Altogether, 219 seizures were found in 41 rats. Of these, 112 were in rats in the MRI and 107 in rats in the EEG cohort. (B) Occurrence of seizures during the long-term video-EEG monitoring that was aimed to start on the 7th post-traumatic brain injury (TBI) month (index month, blue shading). Animal IDs are shown on the y-axis. The time from injury in days is shown on the x-axis. A dashed line separates the MRI and EEG cohorts. Animals from different study sites are shown with different colors (University of Eastern Finland [UEF], blue; Monash University [Monash], orange; University of California, Los Angeles [UCLA], green). The duration of monitoring varied between 2 and 176 days (Table S1). Purple arrows indicate rats in the continuously monitored EEG cohort that already had seizures during the 2nd and 5th post-TBI month. Green arrows indicate animals with seizure clusters (≥3 seizures/24 h).



Monash (44 seizures, 87 ± 41 s, median = 82 s, range = 23–180 s), and UCLA (62 seizures, 108 ± 73 s, median = 101 s, range = 15–390; $p > .05$).

The average seizure duration per rat was 100 ± 50 s (40 rats, median = 95 s, range = 21–254 s; Figure 5E,F). The average seizure duration did not differ between UEF (15 rats, 104 ± 48 s, median = 97 s, range = 21–180 s), Monash (15 rats, 76 ± 37 s, median = 73 s, range = 25–135 s), and UCLA (10 rats, 129 ± 58 s, median = 132 s, range = 57–254; $p > .05$).

3.4.3 | Behavioral severity of electrographic seizures

Racine score was available for 58% (128/219) of the seizures. No videos were available at UCLA for Racine scoring. The average Racine score was 3.34 ± 1.94 (median = 5.00, 128 seizures). Of the 128 seizures, 9% (11/128) had score 0, 22% (28/128) score 1, 7% (9/128) score 2, 3% (4/128) score 3, 9% (11/128) score 4, and 51% (65/128) score 5. The average Racine score of all seizures was greater at UEF (3.69 ± 1.81, median = 5.00, 89 seizures) as compared to that at Monash (2.54 ± 2.00, median = 1.00, 39 seizures; $p < .001$).

The average Racine score per rat was 2.72 ± 2.04 (median = 3.20, 30 rats; Figure 5G,H). The average Racine score per rat did not differ between UEF (3.15 ± 1.99, median = 3.85, 16 rats) and Monash (2.24 ± 2.06, median = 1.00, 14 rats; $p > .05$).

3.4.4 | Seizure clusters

Of the 219 seizures, 53% (117/219) occurred as part of a seizure cluster (≥3 seizures/24 h) and 9% (19/219) as part of SE (Figure S3). The remaining 38% (83/219) of the seizures were more isolated. As shown in Figure 4B, 42% (10/24) of the rats with epilepsy in the MRI cohort and 29% (5/17) in the EEG cohort had one or more seizure clusters ($p > .05$). There were no site differences in the occurrence of seizure clusters ($p > .05$).

3.4.5 | Seizure occurrence during lights-on/lights-off periods

Of the 209 seizures with data available, 56% (116/209) occurred during lights-on period and 44% (93/209) during lights-off period ($p > .05$; Figure S4). There were no differences between the EEG and MRI cohorts ($p > .05$). Also, there were no site differences when data from the EEG or the MRI cohorts were analyzed separately ($p > .05$).

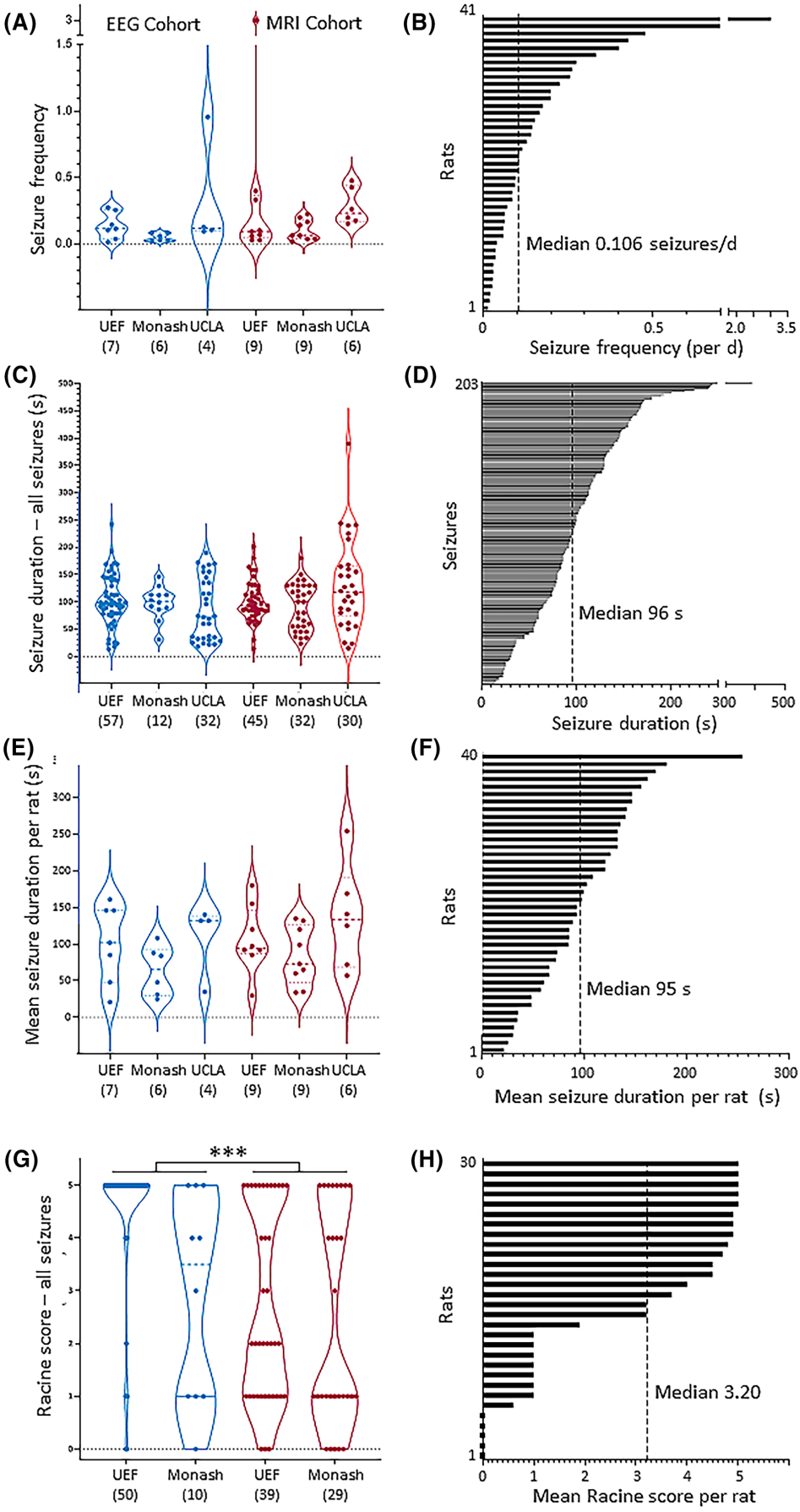
3.4.6 | Seizure onset electrode

We were able to locate the seizure onset electrode in 39% (86/219) of the seizures (Figure S5). Of the 86 seizures, 97% (83/86) originated ipsilaterally. In 86% (74/86) of seizures, the onset was ipsilateral neocortical and in 10% (9/86) ipsilateral hippocampal. Of the neocortical seizures with ipsilateral onset, 54% (40/74) originated caudal to craniotomy in the epidural O1 or intracortical Po1/Po3 electrodes.

3.4.7 | Seizure propagation

Seizures were considered fast-propagating if they spread from the seizure onset electrode (if it could be determined) to another (either ipsilateral or contralateral) within 3 s. Three propagation patterns were categorized:

FIGURE 5 Seizure characteristics. (A) Violin plots showing the average seizure frequency (seizures/24 h) in each animal in the electroencephalography (EEG) and magnetic resonance imaging (MRI) cohorts at different study sites. Each dot refers to one animal. No differences were found between the EEG and MRI cohorts when all animals from different study sites were combined or different sites were analyzed separately (all $p > .05$). Number of animals is in parentheses. (B) Distribution of average seizure frequencies (seizures/monitoring days) in 41 rats with posttraumatic epilepsy (PTE). The median seizure frequency (dashed line) was .106 seizures/day. (C) Duration of individual seizures in the EEG and MRI cohorts at different study sites. No differences were found between the EEG and MRI cohorts when all animals from different study sites were combined or different sites were analyzed separately (all $p > .05$). Number of seizures is in parentheses. (D) Distribution of the duration of 208 electrographic seizures. The median seizure duration was 96 s. (E) Average seizure duration in each animal in the EEG and MRI cohorts at different study sites. No differences were found between the EEG and MRI cohorts when all animals from different study sites were combined or different sites were analyzed separately (all $p > .05$). (F) Distribution of average seizure duration in 40 rats with PTE. (G) Average Racine score was greater in the EEG (60 seizures) than MRI (68 seizures) cohort (4.2 ± 1.6 vs. 2.6 ± 1.9; Mann–Whitney *U*). When both sites were analyzed separately, no differences between the cohorts were found ($p > .05$). Number of scored seizures is in parentheses. (H) Distribution of average Racine score in 30 rats with PTE. The median Racine score was 3.2. Monash, Monash University; UCLA, University of California, Los Angeles; UEF, University of Eastern Finland.



generalized (no focal onset could be defined), fast propagation, and slow propagation. We were able to define seizure propagation in 79% (174/219) of the seizures (Figure S6). Of the 174 seizures, 66% (114/174) were fast propagating, 17% (29/174) slow propagating, and 18% (31/174) generalized.

3.5 | Magnetic resonance imaging

Figure S7 shows the coronal in vivo T2-weighted MRIs of each rat with epilepsy in the MRI cohort and coronal ex vivo T2*-weighted multiecho gradient echo MRIs in the EEG cohort. In each case, the impact-related lesion was visible and its epicenter was located in the auditory association cortex.

4 | DISCUSSION

The major objective of EpiBioS4Rx Project 1 is to identify translational diagnostic and prognostic plasma, EEG, and MRI biomarkers for posttraumatic epileptogenesis in a rat model of PTE induced with lateral FPI. To achieve adequate statistical power to discover a biomarker with area under the curve = .700, the project was performed with a multicenter design involving three centers: UEF in Finland, Monash in Australia, and UCLA in the USA. Our first task was to assess whether the lateral FPI-induced model of PTE was similarly produced at all three study sites. Specifically, the following questions were addressed: is there a difference in the (1) prevalence of epilepsy or (2) epilepsy phenotype between the centers and (3) do the procedural differences at different study sites affect epilepsy outcome? As the data show, the lateral FPI rat model of PTE is highly reproducible. The prevalence and epilepsy phenotype did not differ between centers despite some procedural differences.

4.1 | Prevalence of PTE did not differ between sites or between MRI and EEG cohorts

In the present study, 22% of the rats with lateral FPI-induced severe TBI developed epilepsy, that is, had at least one spontaneous seizure during the 7th month video-EEG recording, in accordance with the International League Against Epilepsy definition of PTE.[18] Previous studies by the UEF team demonstrated a 11% (7/65) epilepsy rate after 3–4 months, a 22%–29% epilepsy rate after 6–7 months, and a 46% epilepsy rate after 12 months in adult male Sprague Dawley rats that had experienced a lateral FPI.[14,19,20] Accordingly, the Monash team had previously reported a 30% (7/23) PTE rate 6 months after lateral FPI in adult male Wistar rats.[15] The UCLA team had previously reported a 25% (4/12) epilepsy rate in adult male Sprague Dawley rats EEG monitored for up to 4 months.[21] Although the cohort sizes and video-EEG monitoring periods in previous studies have been variable, affecting the sensitivity of detecting seizures, occurring at a low frequency and in a low proportion of animals, the epilepsy prevalence has been remarkably similar between the studies at the 6 months post-TBI time point. Importantly, 80% (33/41) of the rats with a first seizure also had a second seizure during the follow-up, justifying epilepsy diagnosis after the first unprovoked seizure.[18]

Did isoflurane anesthesia affect posttraumatic epileptogenesis? In the MRI cohort, rats underwent five long procedures under anesthesia, including a surgery-related, four MRI-related, and an electrode implantation-related anesthesia. By contrast, in the EEG cohort, rats were exposed to one long anesthesia session that involved the surgery for injury followed by electrode implantation. In addition, both cohorts were exposed to four shorter sedations during post-TBI tail vein blood sampling. Interestingly, the epilepsy rate in the whole MRI cohort was 27% and in the EEG cohort 18%, suggesting no antiepileptogenic effect of repeated isoflurane anesthesia. This is in correlation with our previous study, showing no effect of MRI-related isoflurane anesthesia duration on the prevalence of PTE.[22] Also, our data show that the presence of chronically implanted intracerebral and epidural electrodes did not increase the prevalence of epilepsy.

The prevalence of epilepsy tended to be higher in the MRI than the EEG cohort at both UEF (28% vs. 16%) and Monash (29% vs. 16%), whereas at UCLA the epilepsy rate did not differ (22% vs. 24%) between the cohorts. This can reflect the "normal" variability in epilepsy prevalence after lateral FPI, when analysis is done by comparing smaller subcohorts. However, the tendency toward lower epilepsy prevalence in the UCLA MRI cohort can also relate to a shorter 7th month EEG monitoring time as compared to that at UEF or Monash.

Epileptogenesis is a "moving target," with an increase in prevalence over time. Consequently, its detection rate is time dependent. Our data emphasize the need for standardization of the intersite and intercohort video-EEG follow-up timing and duration to optimize the detection of PTE at a given follow-up point without recording bias. As we will summarize in section 4.4, the unforeseen procedural obstacles can challenge the standardization efforts. Availability of early prognostic biomarkers for PTE will likely reduce the need of long-term monitoring, and overall, reduce the proportion of

false negative cases in the study cohort at a given analysis time period.

4.2 | Epilepsy phenotype did not differ between the sites or between MRI and EEG cohorts

At all study sites, a large majority of the seizures had a perilesional cortical onset. At UEF, 24% of the seizures had an ipsilateral hippocampal onset, which was not detected at Monash or UCLA. One explanation is that at UEF all TBI animals were monitored with a 12-channel montage, including a hippocampal electrode. According to histology, 93% (70/75) of the electrode tips in UEF rats with TBI were in the hippocampus proper or the dentate gyrus.[23] However, at Monash, only 76% (52/68) of the TBI animals were recorded with hippocampal electrodes, six of which required reimplantation with epidural electrodes only. Consequently, in only 44% (30/68) of the TBI cases could ex vivo MRI confirm the location of the electrode tip in the hippocampus or the dentate gyrus. At UCLA, all animals were recorded with a 12-channel montage, but due to a high reimplantation rate (16/44) and missing ex vivo MRIs (6/44), a correct positioning of the hippocampal electrodes could be confirmed in only 18% (8/44) of the TBI cases in ex vivo MRI. It should be noted that a correct hippocampal electrode location found in ex vivo MRI could be validated in histology in 87% of the cases.[23] Taken together, the lack of hippocampal seizure onset at Monash and UCLA could relate to a lower number of animals recorded with hippocampal electrodes.

It is important to note that the seizure onset could be defined in only 40% of the seizures recorded. The analysis was challenged by a limited number of recording electrodes as well as by bad electrode connections and poor EEG quality at the early phase of the study. Also, many unprovoked seizures appeared generalized or showed a very fast propagation. Importantly, the seizure frequency, seizure duration, and behavioral seizure severity did not differ between the three study sites. Of the seizures, 53% were part of the seizure cluster, which were found at all sites. As reported previously, seizures occurred quite equally during lights-on and lights-off periods.[14]

There were no phenotypic differences between the MRI and EEG cohorts despite rather different study designs. At Monash, however, none of the 12 seizures recorded in the EEG cohort occurred within a seizure cluster.

Analysis of the seizure-related behavioral symptoms in UEF and Monash recordings indicated that 9% of electrographic seizures did not have a behavioral correlate and had been unrecognized without EEG. Moreover, 32% of the seizures with behavioral symptoms were classified as focal with Racine score 1–2. Overall, these data suggest that without EEG recording there is a risk of underdiagnosing PTE after lateral FPI.

Taken together, like the prevalence of epilepsy, also the epilepsy phenotype was reproducible between the sites. The occurrence of seizure clusters together with an overall low seizure frequency needs to be taken into consideration when planning the treatment trials and defining, for example, the 50% responder rate.

4.3 | Some variability in animal and TBI-induction related factors did not affect epileptogenesis

Genetic background can affect the risk of structural epileptogenesis after TBI as shown in mice, the CD1 or APP/PS1 mice being more sensitive to posttraumatic epileptogenesis as compared to B6 mice after controlled cortical injury.[24–29] Here, all study sites used adult male Sprague Dawley rats, although from different vendors.

Also, there were minor differences in pellets used for feeding, use of antibiotics, use of a drill versus handheld trephine for craniectomy, and/or surgery-related analgesics. As the prevalence and epilepsy phenotype did not differ between the study sites, we conclude that the model reproducibility can tolerate such procedural variability.

4.4 | Lessons learned

The present study is the first major preclinical attempt to harmonize the production of an animal model across multiple centers for a powered multicenter study on epileptogenesis. All study sites had used the lateral FPI model before initiation of the project. For the needs of EpiBioS4Rx, we made a major attempt to harmonize somewhat variable procedures. We used the same strain, sex, and age of rats as well as aimed at comparable housing, anesthesia, craniectomy site and size, and impactor. We exchanged information on surgical and other procedural details and practiced the procedures. All procedures and their timing were reported using common data elements on an Excel sheet. Finally, we conducted an interim analysis to assess the harmonization at the early stage of the study to identify the action points to improve the performance.[30–36] Considering that the project was undertaken over a period of approximately 3 years, we realized the need of continuing training and monitoring to optimize the project success.

We encountered some obstacles that were not foreseen in project planning. The first one related to malfunction of the pogo-pin electrode connectors at some

time after their implantation. This resulted in exclusion of >40 animals after the 7th month video-EEG monitoring. Moreover, although seizures could still be reliably detected in some animals, the seizure origin could not be analyzed. This experience emphasized the need for a preliminary study to test the materials planned to be used in the final study.

Another challenge related to the presence of brain lesions detected in MRI at later stages of the study, including surgery-related brain lesions and abscesses. In the MRI cohort, the surgery-related lesions could be detected early in in vivo MRIs and the animals were excluded. In the EEG cohort, however, the ex vivo MRI became available at the end of the study, that is, after a 7-month follow-up, including the labor-intensive video-EEG monitoring. These observations emphasize the benefits of the use of structural MRI, if available, for early screening of the animal cohort to stratify only the animals with TBI-related brain lesions into the follow-up. Moreover, either MRI or histology should be performed to exclude non-TBI-related structural epilepsy in TBI animals. Structural analysis is also important to verify the depth electrode positioning for analysis of signal origin.

5 | CONCLUSIONS

The lateral FPI-induced epileptogenesis and epilepsy phenotype are highly reproducible across sites if harmonization of methodology and data collection is undertaken. Consequently, the model is suitable for a multicenter design, which may be needed to produce larger animal numbers for a statistically powered biomarker study and/or a treatment trial. However, a preliminary study, assessing the harmonization of the procedures and testing the materials to be used, is highly recommended. Also, the constant training and monitoring of the progress at different study sites should be anticipated and included in the budgeting for the study. Further studies are needed to explore the model reproducibility in females and different age groups.

AUTHOR CONTRIBUTIONS

Asla Pitkänen, Terence J. O'Brien, and Richard Staba designed the study. Xavier Ekolle Ndode-Ekane, Idrish Ali, Cesar Santana-Gomez, Pablo Casillas-Espinosa, Pedro Andrade, Tomi Paananen, Noora Puhakka, Gregory Smith, Neil Harris, Nigel C. Jones, Sandy R. Shultz, and Matt Hudson set up the methodologies. Xavier Ekolle Ndode-Ekane, Idrish Ali, Cesar Santana-Gomez, Pablo Casillas-Espinosa, Juliana Silva, Emma Braine, Glen R. Yamakawa, and Rhys D. Brady performed the experiments. Xavier Ekolle Ndode-Ekane, Idrish Ali, Cesar Santana-Gomez, and Pablo Casillas-Espinosa analyzed the procedures-related and physiological data. Pedro Andrade, Idrish Ali, Cesar Santana-Gomez, Tomi Paananen, Rhys D. Brady, Matt Hudson, Richard Staba, and Asla Pitkänen analyzed the EEG data. Riikka Immonen, Eppu Manninen, Gregory Smith, Neil Harris, David K. Wright, and Olli Gröhn analyzed the MRI data. Asla Pitkänen, Xavier Ekolle Ndode-Ekane, Idrish Ali, Cesar Santana-Gomez, Pedro Andrade, Riikka Immonen, Olli Gröhn, Richard Staba, and Terence J. O'Brien compiled the data and wrote the manuscript.

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CONFLICT OF INTEREST STATEMENT

None of the authors has any conflict of interest to disclose. 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.

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SUPPORTING INFORMATION

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

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