FULL-LENGTH ORIGINAL RESEARCH

Postinjury weight rather than cognitive or behavioral impairment predicts development of posttraumatic epilepsy after lateral fluid-percussion injury in rats

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Abstract
Objective: To identify postinjury physiologic, behavioral, and cognitive biomarkers for posttraumatic epilepsy to enrich study populations for long-term antiepileptogenesis studies.

Methods: The EPITARGET cohort with behavioral follow-up and 1-month 24/7 video-electroencephalography (vEEG) monitoring included 115 adult male Sprague-Dawley rats with lateral fluid-percussion–induced traumatic brain injury (TBI), 23 sham-operated controls, and 13 naive rats. Animals underwent assessment of somatomotor performance (composite neuroscore), anxiety-like behavior (elevated plus maze, open field), spatial memory (Morris water maze), and depression-like behavior (Porsolt forced swim, sucrose preference). Impact force, postimpact apnea time, postimpact seizure-like behavior, and body weight were monitored.

Results: TBI rats were impaired in the composite neuroscore ($P < .001$) on days (D) 2-14 and in the spatial memory test ($P < .001$) on D35-39 post-TBI. Differences in the elevated plus-maze (D28 and D126) and in the open field (D29 and D127) between TBI rats and controls were meager. No differences were observed in the Porsolt forced swim and sucrose preference tests as compared with sham-operated controls. Epilepsy developed in 27% of rats by the end of the sixth month. None of the behavioral or cognitive outcome measures discriminated rats with or without epilepsy. The receiver-operating characteristic analysis indicated that a decrease in body weight between D0 and D4 differentiated TBI rats with epilepsy from TBI rats without epilepsy (48% sensitivity, 83% specificity, area under the curve [AUC] 0.679, confidence interval [CI] 95% 0.56-0.80, $P < .01$). A 16% body weight decrease during D0-D4 could be used as a biomarker to enrich the study population from 27% (observed) to 50%.

Significance: Single behavioral and cognitive outcome measures showed no power as prognostic/diagnostic biomarkers for posttraumatic epilepsy. A reduction in body weight during the first postinjury week showed some prognostic value for
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 (www.center-tbi.eu/) and the United States (www.cdc.gov/traumaticbraininjury) experience TBI. Depending on the location, type, and severity, TBI can result in different types of cognitive, emotional, and behavioral comorbidities that may not be readily apparent, but progress in “silence.”\(^2,3\) TBI is also a major etiology of epilepsy in humans, and it is estimated that the risk of posttraumatic epilepsy (PTE) increases approximately 16-fold after severe TBI and 10% to 20% of acquired structural epilepsies are due to TBI.\(^4,6\)

Epileptogenesis refers to the development and extension of tissue capable of generating spontaneous seizures, resulting in (a) the development of an epileptic condition and/or (b) progression of the epilepsy after it is established.\(^7\) Like many neuropsychiatric comorbidities, concomitant epileptogenesis can remain “silent” for months to years after TBI.\(^3\) In fact, epidemiologic studies indicate that ~80% of TBI patients who eventually develop epilepsy will have the PTE diagnosis within 2 years after TBI.\(^4,5\) Brain epileptogenic networks in patients with structural epilepsies typically involve the hippocampus, amygdala, temporal/frontal cortex, and thalamus.\(^8\) Recent electrophysiologic and functional imaging data in both experimental models and human epilepsy show that epilepsy is a network disorder, even when the seizure onset is focal.\(^9,10\) It is important to note that pathology in the most epileptogenic brain areas and their connectivity has also been linked to the evolution of neuropsychiatric and behavioral comorbidities, generating a hypothesis that the comorbidogenic and epileptogenic networks overlap.

PTE is considered an epilepsy syndrome suitable for anti-epileptogenesis studies.\(^11\) TBI is common, PTE is relatively frequent after severe TBI, and the onset of epileptogenesis (ie, time of impact) is typically known. Animal models of TBI recapitulate many components of the pathology, including epilepsy and behavioral phenotypes.\(^12\) The most extensively investigated PTE model is epileptogenesis after lateral fluid percussion injury (FPI). FPI triggers epileptogenesis in approximately 25% to 50% of rats over a 12-month follow-up.\(^13-18\) It is well-documented that lateral FPI also results in poor performance in spatial memory, depression, and anxiety tests,\(^19-22\) which persists for months after FPI.\(^21\) As observed in humans with severe TBI, structural lesions after FPI-induced TBI are progressive and widely distributed, including in the cerebral cortex, thalamus, hippocampus, and amygdala, and their connections.\(^23\) Thus, like in humans, there is a spatiotemporal overlap in the evolution of epileptogenic and other comorbidogenic brain networks in the lateral FPI model.

Despite almost 50 favorable preclinical proof-of-concept trials in animal models, including about a dozen trials performed in models of PTE, there are no treatments for patients at risk for epileptogenesis after TBI or any other etiology.\(^11\) The major obstacle for the development of therapy is the lack of biomarkers that could be used to enrich patient populations for anti-epileptogenesis studies and predict treatment efficacy at an early stage of treatment.\(^11,24\) Two recent studies suggest that behavioral and cognitive abnormalities could serve as prognostic biomarkers for epileptogenesis after status epilepticus.\(^25,26\) The present study was designed to test the hypothesis that behavioral abnormalities often preceding the epilepsy diagnosis will present as diagnostic biomarkers for ongoing epileptogenesis in an animal model of PTE. The data derive from the analysis of a large EPITARGET cohort of 13 naive, 23 sham-operated experimental controls, and 115 rats with severe lateral FPI
that completed epilepsy phenotyping by 1-month-long video-electroencephalography (EEG) monitoring, as well as behavioral follow-up performed at different time points of epileptogenic process, focusing on motor deficits assessed with a composite neuroscore, anxiety with elevated plus-maze and open field, spatial memory assessed with the Morris water maze, and depression-like behavior assessed with sucrose preference and Porsolt forced-swim tests.

2 | METHODS

2.1 | Study design and animal groups

The study design is summarized in Figure 1. To facilitate systematic data collection, all animal and procedure-related information was collected using common data elements and case report forms tailored for the EPITARGET project (www.epitarget.eu), and stored in a secure web-based REDCap (Research Electronic Data Capture) database at the University of Eastern Finland server.

Detailed methodologic descriptions are given in Appendix S1. Adult male Sprague-Dawley rats (n = 257) were randomized into three groups (Figure 2): (a) rats with lateral FPI injury (TBI, n = 214), (b) sham-operated experimental controls (Sham, n = 27), and (c) naive animals (Naïve, n = 16).

Impact force, postimpact apnea time, postimpact seizure-like behavior, and body weight were monitored. None of the rats showed handling-related seizures on the day of behavioral testing. Occurrence of epileptic seizures was monitored during the sixth postinjury month by 24/7 video-electroencephalography (Figure 3).

All the experiments were approved by the animal ethics committee of the Provincial Government of Southern Finland and performed in accordance with the guidelines of the European Community Council Directives 2010/63/EU.

2.2 | Statistical analysis

All data analyses were performed with the investigator blinded to the treatment group. Normal distribution and group variances of behavioral and other parameters measured were tested with a Shapiro-Wilk normality test and Levene test for equality of variance. If the parameter exhibited a skewed distribution and unequal variances between the groups, the differences between the animal groups were
evaluated using the Kruskal-Wallis test followed by Dunn post hoc analysis with the Bonferroni-Holm correction for multiple comparisons. Otherwise, one-way analysis of variance ANOVA followed by post hoc analysis with Tukey multiple comparison test was used. Differences between the earlier and later time-points for the elevated plus-maze, open-field, sucrose preference, and Porsolt forced-swim tests were assessed with the paired Wilcoxon signed-rank test. Differences between multiple time points were assessed with the repeated-measures ANOVA followed by the paired t test or with the Friedman test followed by the paired Wilcoxon signed-rank test with the Bonferroni-Holm correction.

Behavioral biomarker performance was evaluated with the receiver-operating characteristic (ROC) curve by calculating the specificity, sensitivity, and area under the ROC curve (AUC). We calculated the 95% confidence intervals (CI95%) for the ROC analysis results with a significance level of 5%. The statistical significance of AUCs was assessed with the Mann-Whitney U test. The optimal cut-off for each parameter
was evaluated with the cutpointr package (v. 1.0.1) for R by maximizing the sum of sensitivity and specificity for the optimal cut-off value.29

The relation between the behavioral parameters, apnea, impact pressure, and duration of acute postimpact seizure-like behavior was assessed with the Spearman rank correlation test. The difference was considered significant if \( P < .05. \) All statistical analyses were performed using R (v. 3.5) with RStudio (v. 1.1.383).

3 RESULTS

3.1 Development of epilepsy

Late unprovoked spontaneous electrographic seizures were observed in 27% (31/115) of the rats with TBI during the 1-month video-electroencephalography (video-EEG) monitoring at the sixth post-TBI month. Furthermore, the biomarker was expected to have an area under the curve > 0.700 in the receiver-operating characteristic (ROC) analysis. Consequently, we needed at least 22 rats with epilepsy. We expected a 30% acute and 15% follow-up mortality in the TBI group. Altogether, 257 rats were randomized into one of the three groups: 16 naive animals, 27 sham-operated experimental controls, and 214 rats with lateral FPI. Because we were able to monitor approximately 25 rats at the same time in our video-EEG monitoring unit, the rat population was divided into eight successive sub-cohorts. The follow-up of animals was completed over a period of approximately 2 years. The acute postimpact mortality was less than the expected 30%; therefore we excluded the “extra” animals from the follow-up after the maximal size of each sub-cohort was achieved (Exclusions). Fourteen animals had to be killed immediately after the impact due to breakdown of the dura (Killed). In total, 160 rats (14 naive, 24 sham, and 122 TBI) were included in the behavioral follow-up. Altogether, 152 rats that completed the behavioral testing were monitored with video-EEG. Of these 152 rats, one rat died from status epilepticus during the video-EEG monitoring (included in the PTE+ group). The quality of the EEG was poor for one naive animal, and the data for this animal were therefore excluded. Consequently, we had epilepsy phenotypes from 151 of 152 behaviorally tested rats. Of the 115 TBI rats, 31 had epilepsy (one of which had status epilepticus noted as 311). None of the naive or sham-operated experimental controls had epileptic seizures in EEG.

3.2 Mortality, impact pressure, duration of postimpact apnea, and duration of acute postimpact seizure-like behavior

3.2.1 Mortality

Acute postimpact mortality within 48 hours was 15% (32/214) in the TBI group and 4% (1/27) in the sham-operated group. Follow-up mortality within the 6-month study period was 5% (6/122) in the TBI group and 4% (1/24) in the sham-operated group. The causes of death were unknown.

3.2.2 Impact pressure

The mean impact pressure in the TBI group was \( 3.25 \pm 0.10 \) atm (n = 212, median 3.3, range 2.5-3.6 atm). TBI rats with epilepsy (TBI+) were not significantly different from TBI rats without epilepsy (TBI−) (Table 1).

3.2.3 Apnea

The mean duration of postimpact apnea in the TBI groups was 33 ± 17 s (n = 205, median 30.0, range 0-105 s). The TBI+ group was not significantly different from the TBI− group (Table 1).

3.2.4 Postimpact seizure-like behavior

Acute postimpact seizure-like behavior was observed in 22% (46/212) of the rats with TBI, lasting for 28 ± 11 s (median 30, range 5-50 s). The TBI+ group was not significantly different from TBI− group (Table 1).

3.2.5 Correlations

Impact pressure did not correlate with apnea time (\( R = 0.12, P = .025 \)) or duration of acute postimpact seizure-like behavior (\( R = 0.24, P = .083 \)). Similarly, apnea time did not correlate with the duration of acute postimpact seizure-like behavior (\( R = 0.24, P = .059 \)). Impact pressure or apnea time did not correlate with any of the measured behavioral outcome measures. The longer the duration of acute postimpact seizure-like behavior,
TABLE 1 Impact, postimpact parameters, and behavioral and cognitive performance of different animal groups during early epileptogenesis after traumatic brain injury (TBI)

| Outcome | Naive | Sham | TBI All | TBI− | TBI+ |
|---------|-------|------|---------|------|------|
| **Impact and postinjury behavior (D0)** | | | | | |
| Impact pressure (atm) | n.a. | n.a. | 3.25 ± 0.10 (212) | 3.26 ± 0.08 (84) | 3.25 ± 0.08 (31) |
| Duration of acute postimpact apnea (s) | n.a. | n.a. | 33 ± 17 (205) | 33 ± 17 (84) | 34 ± 16 (31) |
| Occurrence of acute postimpact seizure-like behavior | n.a. | n.a. | 46/212 | 12/84 | 6/31 |
| % | n.a. | n.a. | 22% | 14% | 14% |
| Duration (s) | n.a. | n.a. | 28 ± 11 | 26 ± 12 | 27 ± 4 |
| **Composite neuroscore (D2, D6, D14)** | | | | | |
| Number of animals | 14 | 23 | 116 | 84 | 31 |
| Baseline | 28.0 ± 0.0 | 28.0 ± 0.0 | 28.0 ± 0.3 | 28.0 ± 0.3 | 28.0 ± 0.0 |
| D2 | 27.9 ± 0.3 | 26.7 ± 1.0 | 8.1 ± 2.3*** | 8.4 ± 2.2*** | 7.5 ± 2.1*** |
| D6 | 27.6 ± 0.6 | 27.1 ± 0.9 | 12.9 ± 3.2*** | 13.2 ± 3.2*** | 12.4 ± 2.9*** |
| D14 | 27.7 ± 0.7 | 27.2 ± 0 | 15.7 ± 3.1*** | 15.8 ± 3.1*** | 15.8 ± 2.5*** |
| **Elevated plus maze (D28)** | | | | | |
| Number of animals | 14 | 23 | 118 | 84 | 31 |
| Total distance (cm) | 1623 ± 277 | 1487 ± 359 | 1453 ± 375 | 1459 ± 367 | 1459 ± 405 |
| Velocity (cm/s) | 5.4 ± 0.9 | 5.0 ± 1.2 | 4.9 ± 1.3 | 4.9 ± 1.2 | 4.9 ± 1.4 |
| Number of entries (per 5 min) | | | | | |
| Total | 23.3 ± 9.3 | 19.4 ± 11.3 | 18.7 ± 9.8 | 19.6 ± 10.6 | 17.0 ± 6.9 |
| Open arms | 1.7 ± 2.4 | 2.4 ± 3.3 | 1.9 ± 4.3 | 2.0 ± 4.6 | 2.0 ± 3.5 |
| Closed arms | 21.6 ± 8.9 | 17.0 ± 9.9 | 16.7 ± 9.3 | 17.5 ± 9.8 | 15.0 ± 7.3 |
| Latency first closed arm entry (s) | 8.1 ± 13.5 | 6.6 ± 15.3 | 20.9 ± 30.4** | 20.5 ± 30.4** | 23.8 ± 36.3* |
| % of open arm entries of all entries | 6.8 ± 10.5 | 9.2 ± 13.0 | 8.7 ± 18.1 | 7.7 ± 16.5 | 12.1 ± 22.2 |
| Time spent (s) in | | | | | |
| Open arms | 3.5 ± 6.9 | 8.6 ± 16.3 | 6.5 ± 25.5 | 3.6 ± 10.8 | 15.0 ± 47.5 |
| Closed arms | 251 ± 29 | 245 ± 43 | 237 ± 52 | 239 ± 49 | 226 ± 59 |
| Center | 42 ± 24 | 44 ± 32 | 54 ± 40 | 54 ± 42 | 57 ± 35 |
| **Elevated plus maze (D126)** | | | | | |
| Number of animals | 11 | 23 | 117 | 84 | 31 |
| Total distance (cm) | 748 ± 333†† † | 794 ± 416†† † | 805 ± 361†† † | 755 ± 314†† † | 971 ± 422†† † |
(Continues)
| Outcome                                | Naive          | Sham           | TBI All        | TBI−           | TBI+           |
|----------------------------------------|----------------|----------------|----------------|----------------|----------------|
| Velocity (cm/s)                        | 2.5 ± 1.1†††   | 2.7 ± 1.4†††   | 2.7 ± 1.2†††   | 2.5 ± 1.0†††   | 3.3 ± 1.4†††   |
| Number of entries (per 5 min)          |                |                |                |                |                |
| Total                                  | 3.5 ± 3.3††    | 5.9 ± 6.9†††   | 4.2 ± 5.1†††   | 3.3 ± 4.0†††   | 6.6 ± 6.8†††   |
| Open arms                              | 0.0 ± 0.0†     | 0.4 ± 1.3†     | 0.0 ± 0.1+†††  | 0.0 ± 0.0+†††  | 0.0 ± 0.2†††   |
| Closed arms                            | 3.5 ± 3.3†††   | 5.5 ± 6.3†††   | 4.2 ± 5.1†††   | 3.3 ± 4.0†††   | 6.6 ± 6.7†††   |
| Latency first closed arm entry (s)     | 2.8 ± 5.7      | 2.2 ± 5.0      | 8.6 ± 25.6†††  | 8.6 ± 28.2†††  | 8.5 ± 17.8†    |
| % of open arm entries                  | 0.0 ± 0.0†     | 2.1 ± 7.1†     | 0.0 ± 0.4+†††  | 0.0 ± 0.0+†††  | 0.1 ± 0.8†††   |
| Time spent (s) in                      |                |                |                |                |                |
| Open arms                              | 0.0 ± 0.0†     | 0.5 ± 0.9†     | 0.1 ± 1.2†††   | 0.0 ± 0.0†††   | 0.0 ± 0.2†††   |
| Closed arms                            | 293 ± 10†††    | 290 ± 14†††    | 288 ± 27†††    | 289 ± 28†††    | 284 ± 22†††    |
| Center                                 | 7 ± 10†††      | 9 ± 12†††      | 12 ± 26†††     | 11 ± 28†††     | 15 ± 21†††     |

Open field (D29)

| Number of animals                      | 14             | 23             | 118            | 84             | 31             |
| Total distance (cm)                    | 2823 ± 807     | 3032 ± 807     | 3039 ± 668     | 2989 ± 977     | 3215 ± 703     |
| Velocity (cm/s)                        | 9.4 ± 2.7      | 10.1 ± 2.2     | 10.2 ± 3.1     | 10.0 ± 3.3     | 10.8 ± 2.4     |
| Number of entries (per 5 min)          |                |                |                |                |                |
| Total                                  | 32.4 ± 25.5    | 34.6 ± 22.3    | 21.4 ± 19.2    | 19.9 ± 18.3*   | 27.0 ± 20.8    |
| Outer zone                             | 13.9 ± 11.7    | 12.8 ± 7.9     | 8.1 ± 7.1      | 7.8 ± 7.0*     | 9.4 ± 7.3      |
| Middle zone                            | 13.9 ± 12.1    | 16.4 ± 11.2    | 9.9 ± 9.7*     | 9.0 ± 9.2*     | 13.0 ± 10.7    |
| Inner zone                             | 4.6 ± 3.8      | 5.4 ± 3.8      | 3.4 ± 3.2      | 3.1 ± 3.0*     | 4.6 ± 3.5      |
| Time spent (s) in                      |                |                |                |                |                |
| Outer zone                             | 256 ± 25       | 257 ± 28       | 264 ± 40       | 265 ± 44       | 259 ± 31       |
| Middle zone                            | 29 ± 17        | 29 ± 21        | 22 ± 34        | 22 ± 38        | 25 ± 22        |
| Inner zone                             | 12 ± 9         | 13 ± 9         | 15 ± 30        | 15 ± 34        | 15 ± 14        |
| Latency (s) first entry                |                |                |                |                |                |
| Outer zone                             | 6.5 ± 4.5      | 6.1 ± 7.4      | 13.6 ± 19.8    | 13.4 ± 19.5    | 15.3 ± 21.1    |
| Middle zone                            | 2.7 ± 2.4      | 2.4 ± 2.3      | 6.6 ± 11.0     | 7.1 ± 10.7     | 5.7 ± 12.1     |
| Inner zone                             | 1.6 ± 5.7      | 1.8 ± 8.3      | 7.0 ± 36.8     | 6.9 ± 40.1     | 8.0 ± 28.3     |

Open field (D127)

| Number of animals                      | 14             | 23             | 117            | 84             | 31             |
| Total distance (cm)                    | 1592 ± 596†††  | 1445 ± 676†††  | 1956 ± 966†††  | 1800 ± 902†††  | 2355 ± 1000††† |
| Velocity (cm/s)                        | 5.3 ± 2.0†††   | 4.8 ± 2.3†††   | 6.5 ± 3.2†††   | 6.0 ± 3.0†††   | 7.9 ± 3.3†††   |
| Number of entries (per 5 min)          |                |                |                |                |                |
| Total                                  | 8.6 ± 8.2†††   | 9.5 ± 14.4†††  | 7.7 ± 11.0†††  | 6.4 ± 7.0†††   | 11.4 ± 17.3††† |
| Outer zone                             | 3.4 ± 3.6†††   | 4.0 ± 6.9†††   | 2.8 ± 4.6†††   | 2.2 ± 2.7†††   | 4.6 ± 7.4†††   |
| Middle zone                            | 3.4 ± 3.6†††   | 4.0 ± 6.9†††   | 2.8 ± 4.6†††   | 2.2 ± 2.7†††   | 4.6 ± 4.7†††   |
| Inner zone                             | 1.9 ± 1.5      | 1.4 ± 1.1†††   | 2.0 ± 3.6†††   | 2.0 ± 3.9†††   | 2.2 ± 2.9†††   |
| Outcome   | Naive | Sham  | TBI All    | TBI− | TBI+ |
|-----------|-------|-------|------------|------|------|
| Time spent (s) in |       |       |            |      |      |
| Outer zone  | 282 ± 19†† | 263 ± 64 | 276 ± 53††† | 276 ± 53††† | 273 ± 56†† |
| Middle zone | 10 ± 12†† | 14 ± 28†† | 8 ± 24††† | 8 ± 27††† | 10 ± 16††† |
| Inner zone  | 7 ± 10 | 22 ± 61† | 15 ± 44††† | 16 ± 41† | 16 ± 53†† |
| Latency (s) first entry |       |       |            |      |      |
| Outer zone  | 6.7 ± 8.6 | 27.1 ± 64.2 | 11.4 ± 26.2††† | 13.9 ± 30.2† | 5.3 ± 7.1††† |
| Middle zone | 5.5 ± 8.5 | 21.5 ± 61.7 | 7.5 ± 18.9† | 8.8 ± 21.7 | 4.7 ± 7.8 |
| Inner zone  | 6.6 ± 20.8 | 0.0 ± 0.0 | 2.3 ± 16.9 | 0.8 ± 7.1 | 6.4 ± 30.2 |

**Sucrose preference (D29-32)**

| Number of animals | 14 | 23 | 117 | 83 | 31 |
|--------------------|----|----|-----|----|----|
| Consumption (g)    |    |    |     |    |    |
| Sucrose            | 91 ± 24 | 100 ± 32 | 100 ± 41 | 101 ± 43 | 99 ± 39 |
| Water              | 8 ± 2 | 11 ± 8 | 14 ± 10 | 13 ± 9 | 15 ± 11 |
| Total              | 100 ± 24 | 111 ± 31 | 113 ± 38 | 114 ± 39 | 114 ± 38 |
| % sugar            | 91 ± 5 | 89 ± 11 | 86 ± 12 | 87 ± 12 | 86 ± 12 |

**Sucrose preference (D127-130)**

| Number of animals | 14 | 23 | 115 | 84 | 31 |
|--------------------|----|----|-----|----|----|
| Consumption (g)    |    |    |     |    |    |
| Sucrose            | 86 ± 26 | 84 ± 28†† | 86 ± 12††† | 84 ± 33††† | 87 ± 32† |
| Water              | 9 ± 3 | 11 ± 5 | 13 ± 7 | 13 ± 7 | 12 ± 6 |
| Total              | 96 ± 24 | 94 ± 25††† | 97 ± 31††† | 97 ± 32††† | 99 ± 31†† |
| % sugar            | 89 ± 7 | 88 ± 8 | 86 ± 10† | 85 ± 10† | 87 ± 8 |

**Porsolt forced swim (D42)**

| Number of animals | 14 | 23 | 118 | 84 | 31 |
|--------------------|----|----|-----|----|----|
| Duration (s)       |    |    |     |    |    |
| Swimming           | 100 ± 43 | 99 ± 41 | 106 ± 40 | 110 ± 38 | 100 ± 44 |
| Climbing           | 54 ± 29 | 50 ± 25 | 68 ± 31 | 71 ± 32* | 62 ± 29 |
| Immobility         | 146 ± 37 | 150 ± 36 | 126 ± 39* | 120 ± 35** | 139 ± 45 |
| Frequency (per 5 min) |        |        |        |        |        |
| Swimming           | 28.0 ± 5.2 | 26.6 ± 6.0 | 27.4 ± 6.5 | 28.0 ± 6.2 | 26.0 ± 6.3 |
| Climbing           | 13.9 ± 4.3 | 13.1 ± 3.4 | 14.8 ± 5.0 | 15.4 ± 5.0 | 13.5 ± 4.8 |
| Immobility         | 21.6 ± 4.6 | 20.5 ± 6.1 | 21.6 ± 5.4 | 21.7 ± 5.5 | 21.6 ± 5 |
| Latency to immobility (s) | | | | | |

**Porsolt forced swim (D133)**

| Number of animals | 14 | 23 | 117 | 84 | 31 |
|--------------------|----|----|-----|----|----|
| Duration (s)       |    |    |     |    |    |
| Swimming           | 61 ± 24†† | 75 ± 55†† | 80 ± 43††† | 79 ± 42††† | 86 ± 45 |
| Climbing           | 73 ± 41† | 56 ± 45 | 102 ± 53***‡† | 104 ± 55***‡† | 95 ± 47***‡† |
| Immobility         | 166 ± 39 | 170 ± 49† | 118 ± 51*** | 117 ± 52*** | 119 ± 51** |

(Continues)
however, the lower the total neuroscore on D6 ($R = -0.73$, $P = 6 \times 10^{-7}$) and D14 ($R = -0.72$, $P = 1.2 \times 10^{-7}$; data not shown).

### 3.3 | Body weight

The mean weight of TBI animals reduced by 12% on average during the first postinjury week and increased thereafter (Table S1). The mean body remained lower for almost the entire study period compared with that of sham-operated or naive animals (Figure 4A). The mean weight in the TBI+ group was significantly lower than that in the TBI− group on D3-D84 post-TBI ($P < .05$; Table S1).

The ROC analysis indicated that body weight (g) discriminated the TBI+ group ($n = 31$) from the TBI− group ($n = 84$) on D4 ($P < .001$), D6 ($P < .05$), D7 ($P < .05$), D14 ($P < .001$), and D35 ($P < .0001$) (Table S1).

ROC analysis indicated that percent weight loss between D0 and later time-points discriminated the epileptic animals from nonepileptic animals on D0-D4 (48% sensitivity, 83% specificity, AUC 0.679, CI 95% 0.56-0.80, $P < .01$; Figure 4C-D), D0-D6 (52% sensitivity, 82% specificity, AUC 0.662, CI 95% 0.55-0.78, $P < .01$), D0-D7 (83% sensitivity, 49% specificity, AUC 0.684, CI 95% 0.57-0.79, $P < .01$), D0-D14 (59%
**FIGURE 3** Epilepsy phenotyping. A, Electrode placement. Location of the craniotomy (light green circle) and three epidural recordings (blue; C3, CP4, O1), and reference (green; Ref) and ground (orange; G) electrodes in the skull. Grid dimensions 1 mm × 1 mm. B, A representative coronal brightfield photomicrograph of a thionin-stained section of a rat with lateral fluid-percussion injury 6 months earlier. Open arrow points to the lesion epicenter. Note the thalamic atrophy and ventricle enlargement ipsilaterally. Abbreviations: HC, hippocampus; Th, thalamus; v, lateral ventricle. C, A representative example of a secondarily generalized seizure on electroencephalography (EEG). Letters on the left refer to the electrode placements shown in panel A. Yellow bars indicate the beginning and end of the seizure. Note that the seizure started at the N3-REM (rapid-eye-movement) transition and was followed by postictal attenuation in EEG.

**FIGURE 4** Body weight follow-up during the 6-month study period. (A) Post-traumatic brain injury (TBI) evolution of body weight loss and gain in different animal groups. The upper part of panel A shows the mean weight (± standard error of the mean) in grams of each animal group at different time-points after TBI or sham operation (122 TBI, 24 sham, 14 naive). The dot-plot in the lower part of panel A shows the percent weight decline in each TBI animal compared with that at baseline (see Appendix S1 for statistics). (B) Violin plot shows the distribution of body weight in different treatment groups on day (D) 4 after TBI or sham injury. Note a high variability in body weight in the TBI group compared with that in the naive or sham-operated animals. Rat with epilepsy (TBI+) had a lower body weight compared with rats without epilepsy (TBI−). (C) A graphical presentation of optimal cut-off analysis and corresponding ROC curve. Dashed line indicates the optimal cut-off (16%). ROC analysis indicates that a decrease (shown as a percentage) in body weight between D0 and D4 differentiated the TBI+ from TBI− rats (48% sensitivity, 83% specificity, AUC 0.679, CI 95% 0.56-0.80, P < .01). (D) Summary of true-positive (TP), false-negative (FN), false-positive (FP), and true-negative (TN) values obtained in the optimal cut-off analysis. Note that a 16% body-weight decrease on D4 was able to enrich the study population from 27% to 50% (14 TP, 14 FP). (E) Flow chart of animal numbers, when the D0-D4 optimal cut-off value was applied to the study population with a 27% epilepsy rate. After excluding “negative cases” with D0-D4 weight drop <16% (FN, TN), 14 of the 28 rats (50%) had epilepsy. Abbreviations: AUC, area under curve; BL, baseline (before injury); CI, confidence interval; D, day; N, negative; P, positive; ROC, receiver-operating characteristic (curve). Statistical significance: *P < .05; **P < .01; ***P < .001 (group differences were calculated with Kruskal-Wallis followed by Dunn post hoc analysis with Bonferroni-Holm correction for multiple comparisons; AUC statistics with Mann-Whitney U test).
sensitivity, 76% specificity, AUC 0.701, CI 95% 0.59-0.81, 
P < .001), and D0-D35 post-TBI (81% sensitivity, 46% spec-
ificity, AUC 0.657, CI 95% 0.55-0.77, P < .01).

We then calculated whether acute body weight loss could 
be used to select for those animals likely to develop epilepsy 
in order to enrich the study population for long-term fol-
low-up. The optimal cut-point analysis indicated that a 16% 
weight loss between D0 and D4 increased the percentage of 
rats with epilepsy in the study population from the expected 
27% to 50% (Figure 4E).
3.4 | Neuroscore

Performance in the composite neuroscore test was evaluated before the impact, and thereafter on D2, D6, and D14 postinjury. The data are summarized in Table 1.

3.4.1 | Sham-operated experimental controls vs naive rats

The performance of sham-operated control group was comparable to that of naive rats (Table 1).

3.4.2 | TBI vs experimental sham-operated controls

On D2, D6, and D14, the total composite neuroscore was lower in rats with TBI than in the sham-operated controls or naive rats ($P < .001$, Table 1).

3.4.3 | TBI with (TBI+) and without (TBI−) epilepsy

The total neuroscore did not differ between the TBI+ and TBI− animals on any testing day (Table 1).

The ROC analysis indicated that performance in the neuroscore test (total score or various components [data not shown]) did not differentiate the TBI+ and TBI− groups (AUC <0.70, Table 2).

3.5 | Elevated plus-maze

Performance on both testing days (D28 and D126) is summarized in Table 1.

3.5.1 | Sham-operated experimental controls vs naive rats

The performance of sham-operated control group was comparable to that of naive rats (Table 1).

3.5.2 | TBI vs sham-operated experimental controls

On D28, TBI animals had a longer latency to the first entry into the closed arm than sham-operated control animals ($P < .01$; Table 1). On D126, TBI animals made fewer open

| Parameter | AUC | CI95% |
|-----------|-----|------|
| Impact pressure (atm) | 0.553 | 0.438-0.668 |
| Duration of postimpact apnea (s) | 0.507 | 0.381-0.632 |
| Duration of acute postimpact seizure-like behavior (s) | 0.603 | 0.360-0.845 |
| Composite neuroscore (D2, D6, D14) | | |
| D2 | 0.625 | 0.508-0.742 |
| D6 | 0.575 | 0.460-0.689 |
| D14 | 0.517 | 0.406-0.629 |
| Elevated plus maze (D28) | | |
| Total distance | 0.507 | 0.388-0.626 |
| Velocity | 0.508 | 0.389-0.628 |
| Number of entries | | |
| Total | 0.560 | 0.454-0.666 |
| Open arm | 0.566 | 0.459-0.673 |
| Closed arm | 0.564 | 0.454-0.675 |
| Latency first closed arm entry | 0.512 | 0.389-0.635 |
| % of open arm entries | 0.587 | 0.476-0.697 |
| Time spent in | | |
| Open arm | 0.604 | 0.491-0.717 |
| Closed arm | 0.572 | 0.457-0.686 |
| Center | 0.539 | 0.426-0.653 |
| Open field (D29) | | |
| Total distance | 0.548 | 0.440-0.657 |
| Velocity | 0.551 | 0.442-0.660 |
| Number of entries | | |
| Total | 0.614 | 0.497-0.732 |
| Outer zone | 0.587 | 0.471-0.702 |
| Middle zone | 0.617 | 0.499-0.735 |
| Inner zone | 0.634 | 0.513-0.754 |
| Time spent in | | |
| Outer zone | 0.597 | 0.483-0.709 |
| Middle zone | 0.615 | 0.502-0.728 |
| Inner zone | 0.634 | 0.513-0.754 |
| Latency 1st entry | | |
| Outer zone | 0.529 | 0.409-0.649 |
| Middle zone | 0.515 | 0.402-0.628 |
| Inner zone | 0.514 | 0.452-0.576 |
| Sucrose preference (D29-32) | | |
| Consumption | | |
| Sugar | 0.501 | 0.382-0.620 |
| Water | 0.481 | 0.354-0.609 |
| Total | 0.494 | 0.373-0.615 |
| % sugar | 0.515 | 0.395-0.639 |

(Continues)
arm entries ($P < .05$) and spent less time in the open arms ($P < .05$) than sham-operated animals (Table 1).

### 3.5.3 TBI with (TBI+) and without (TBI−) epilepsy

Performance of TBI+ and TBI− animals did not differ in any of the elevated plus-maze outcome measures (Table 1).

### 3.5.4 D28 vs D126

All animal groups exhibited a significant decrease in activity (eg, reduced speed, reduced number of entries) in the elevated plus maze from D28 to D126 (Table 1).

### 3.6 Open Field

Performance on both testing days (D29 and D127) is summarized in Table 1.

#### 3.6.1 Sham-operated experimental controls vs naive rats

The performance of the sham-operated control group was comparable to that of naive rats on both testing days ($P > .05$, Table 1).

#### 3.6.2 TBI vs sham-operated experimental controls

On D29, rats with TBI visited the middle zone less frequently than the sham-operated controls ($P < .05$; Table 1). On D127, there were no significant differences between the groups (Table 1).

#### 3.6.3 TBI with (TBI+) and without (TBI2212) epilepsy

The performance of TBI+ and TBI− animals in the open-field test did not differ in any of the outcome measures on D29 or D127 (Table 1).

#### 3.6.4 D29 vs D127

All animal groups exhibited a significant decrease in activity (eg, reduced speed, reduced number of entries) in the open-field test from D29 to D127 (Table 1).

The ROC analysis indicated that performance in the open-field test did not differentiate the TBI+ and TBI− groups (AUC <0.70, Table 2).

### 3.7 Sucrose preference

Sucrose preference was tested twice (D29-32 and D127-130). The data are summarized in Table 1.
3.7.1 Sham-operated experimental controls vs naive rats

Performance of the sham-operated control group was comparable to that of naive rats on both testing days ($P > .05$, Table 1).

3.7.2 TBI vs sham-operated experimental controls

The two groups had comparable performance on both testing days ($P > .05$, Table 1).

3.7.3 TBI with (TBI+) and without (TBI−) epilepsy

The two groups had comparable performance on both testing days ($P > .05$, Table 1).

3.7.4 D29-32 vs. D127-130

Both the total liquid consumption and consumption of the sucrose solution were slightly reduced in the sham-operated control animals and TBI animals on D127-130 compared with D29-32 ($P > .05$, Table 1).

ROC analysis indicated that performance in the sucrose preference test did not differentiate the TBI+ and TBI− groups (AUC <0.70, Table 2).

3.8 Porsolt forced swim test

Performance on both testing days (D42 and D133) is summarized in Table 1.

3.8.1 Sham-operated experimental controls vs naive rats

The performance of the sham-operated control group was comparable to that of naive rats on both testing days ($P > .05$, Table 1).

3.8.2 TBI vs sham-operated experimental controls

On D42, the TBI group showed a shorter duration of immobility and longer latency to immobility compared with the sham-operated control group (both $P < .05$). On D133, the TBI group exhibited a shorter duration of immobility ($P < .001$) and longer latency to immobility ($P < .05$) compared with the sham-operated control group. In addition, the duration ($P < .001$) and frequency ($P < .05$) of climbing were increased in the TBI group compared with the sham-group.

3.8.3 TBI with (TBI+) and without (TBI−) epilepsy

Both groups had comparable performance on both testing days ($P > .05$, Table 1).

3.8.4 D42 vs D133

Swimming duration was reduced and frequency of immobility increased in all animal groups on D133 compared with that on D42 ($P < .05$). In addition, rats with TBI showed greater climbing duration and higher climbing frequency on D133 compared with D42 ($P < .05$, Table 1).

ROC analysis indicated that performance in the Porsolt forced-swim test did not differentiate the TBI+ and TBI− groups (AUC <0.70, Table 2).

3.9 Morris water maze

Performance in Morris water-maze test (D35-39) is summarized in Table 1 (Kruskal-Wallis followed by Dunnett post hoc analysis).

3.9.1 Sham-operated experimental controls vs naive rats

Performance of the sham-operated control group was comparable to that of naive rats ($P > .05$, Table 1).

3.9.2 TBI vs sham-operated experimental controls

In the TBI group, the path length and the latency to the platform were longer than that in the sham-operated control group on all testing days D1-3 (all $P < .001$; Table 1). The speed of learning or forgetting did not differ between the TBI and sham groups (data not shown).

3.9.3 TBI with (TBI+) and without (TBI−) epilepsy

Performance of TBI+ and TBI− animals did not differ in any of the outcome measures ($P > .05$, Table 1).
LAPINLAMPI et al. during D1-D4 after TBI. Whether the "immediate post-impact seizure-like behaviors" from approximately 30 seconds.13,18,33 It is important to differentiate these "immediate post-impact seizure-like behaviors" from postinjury seizures. We recently demonstrated that almost all animals with lateral FPI develop postinjury nonconvulsive status epilepticus, which is associated with both immediate (<24 hours) electrographic seizures, typically occurring within a few hours after impact, and early (<7 days) electrographic seizures, most of which are nonconvulsive and occur during D1-D4 after TBI.34 Whether the "immediate post-impact seizure-like behavior" associates with electrophysiological characteristics of a seizure, thus belonging to the category of "immediate seizures," remains to be determined, however, despite the technical challenges related to EEG recordings immediately after the impact. Our findings revealed no association between acute seizure-like behavior and development of PTE, consistent with our previous study.18

In the present study, the average body weight reduction during the first postinjury week was 12% in the TBI− group and 15% in the TBI+ group. It is well known that animals with lateral FPI lose weight, which apparently relates to brain injury–induced loss of consciousness and motor impairment, but also to nonconvulsive status epilepticus, which can last for 3-4 days.34 Thereafter, the body weight begins to increase, eventually reaching the level of age-matched sham-operated experimental controls and naive animals. Unexpectedly, we found that the weight loss was more pronounced and the weight gain slower in TBI+ rats than in TBI− rats. A mean weight loss of 16% between postinjury days D0-D4 differentiated the TBI+ and TBI− animals with 48% sensitivity and 83% specificity. We acknowledge that although the AUC of 0.679 was statistically different from the AUC of 0.500, the strength of weight loss as a biomarker for pinpointing the risk of epileptogenesis in individual animals is not high.24 However, the accuracy of finding all the true epileptic cases from all the cases was encouraging, even though a seemingly high false-negative rate. Optimal cut-point analysis suggested that body weight assessment could provide a noninvasive, simple, and affordable method for enriching study populations for chronic preclinical epileptogenesis studies by increasing the anticipated epileptogenesis rate from approximately 27% to 50% in a 6-month follow-up. Whether weight loss is a surrogate marker for the severity of the ongoing seizure activity remains to be investigated. Our data emphasize the need of careful body weight follow-up in the TBI animal cohort over the period of epileptogenesis.

We assessed the anxiety-like behavior of the rats at 1 and 4 months post-TBI using the elevated plus-maze and open-field tests. Previous studies demonstrated that adult rats with moderate to severe lateral FPI show increased anxiety-like behavior in the elevated-plus-maze when assessed at 4 weeks post-TBI, as evidenced by spending less time in the open arms than controls.35 Shultz and co-workers14,35,36 found anxiety-like behavior in rats with lateral FPI at 1 and 3 months, but not at 6 months post-TBI. In our large animal cohort tested at 1 and 4 months post-TBI, the time spent in the open arms did not differ between the TBI, sham-operated, and naive animals, even though the first entry into a closed arm took slightly longer for the TBI rats than sham-operated control rats. Like Shultz et al.,14 we found no difference between the epileptic and nonepileptic animals at any of the time-points. In all animal groups, however, we found a significant reduction in all test parameters at 4 months compared with that at 1 month, which is possibly related to aging, as described previously.37,38 It is also possible that the re-test effect affected the performance in the second test despite of about 3 months interval between the tests.

ROC analysis indicated that performance in the Morris water-maze test did not differentiate the TBI+ and TBI− groups (AUC <0.70, Table 2).

4 DISCUSSION

This study is part of the European Union Framework seven-funded large-scale research project EPITARGET (www.epitarget.eu). Here we report the results of a preclinical single-center biomarker discovery study on PTE. We tested the hypothesis that the severity of behavioral and/or cognitive comorbidities is a prognostic/diagnostic biomarker for posttraumatic epileptogenesis. We also investigated whether the impact-related parameters and postimpact behavior of the rat would be prognostic biomarkers for the development of PTE.

By the end of the sixth month post-TBI, 27% of the rats had developed epilepsy, with an average seizure duration of about 1.5 minutes and a mean seizure frequency of 0.2/day.30,31 Previous studies in humans suggest that the severity of TBI scored using the Glasgow Coma Scale is a major risk factor for posttraumatic epileptogenesis.4,5 In the present study, the lateral FPI-induced impact force varied between 3.09 and 3.43 atm in the TBI+ and 3.03-3.44 in TBI− groups, suggesting that there could be some variability in the severity of TBI. We detected no relationship between the impact force and epileptogenesis, however, in accordance with our previous studies13,18. Moreover, the range of impact forces does not correlate with the severity of the cortical lesion area or T2 signal enhancement in magnetic resonance imaging.31 The lack of correlations likely relates to a relatively narrow range of impact forces administered to the animal cohorts.

Previous human studies suggest that the occurrence of acute seizures is a risk factor for human PTE.4,5 The occurrence of acute "seizures" after lateral FPI based on behavioral observation has been reported for this model.32,33 In correlation with the present study, acute postimpact seizure-like behaviors have been reported to occur immediately after the impact in 20% to 30% of animals, and last for approximately 30 seconds.13,18,33 It is important to differentiate these "immediate post-impact seizure-like behaviors" from postinjury seizures. We demonstrated that almost all animals with lateral FPI develop postinjury nonconvulsive status epilepticus, which is associated with both immediate (<24 hours) electrographic seizures, typically occurring within a few hours after impact, and early (<7 days) electrographic seizures, most of which are nonconvulsive and occur during D1-D4 after TBI.34 Whether the "immediate post-impact seizure-like behavior" associates with electrophysiological characteristics of a seizure, thus belonging to the category of "immediate seizures," remains to be determined, however, despite the technical challenges related to EEG recordings.
Similarly, we found no major effect of lateral FPI on performance in the open-field test. Previously, Kim et al.\textsuperscript{30} and Fucich et al.\textsuperscript{40} assessed rats with lateral FPI at D4 and D7 postinjury, respectively, and found that the injured rats spent less time than controls exploring the center of the open field. Similarly, Schultz et al.\textsuperscript{14} reported that rats with severe lateral FPI spent slightly less time than sham-operated controls in the inner arena at 1 and 3 months, but not at 6 months post-TBI; however, there was no difference between the epileptic and nonepileptic animals. Like in the elevated plus-maze, there was a comparable decrease in activity from 1 month to 4 months in all animal groups, probably related to aging, as demonstrated previously.\textsuperscript{38}

Deficits in hippocampus-dependent spatial learning can be detected as early as 24 hours after lateral FPI, progress over the weeks and months,\textsuperscript{35,41} and associate with reduced blood flow.\textsuperscript{42} Our previous study showed that reduced blood flow in the ipsilateral hippocampus is associated with increased seizure susceptibility at 7 months after lateral FPI.\textsuperscript{43} Consistent with previous studies,\textsuperscript{33,43} our animals exhibited profound impairments in spatial memory. However, performance in the Morris water maze did not differ between the epileptic and nonepileptic animals, which is in agreement with a previous study by Shultz et al.\textsuperscript{14}

Consistent with previous studies, we observed a remarkable impairment in somatomotor performance in the composite neuroscore test in rats with TBI during the first two postinjury weeks.\textsuperscript{33} The speed of movement in other behavioral tests as an indicator of motor performance increased similarly in all animal groups in the order of the elevated plus maze < open field < Morris water maze. Neither the severity of the somatomotor impairment nor the movement speed differentiated the epileptic and nonepileptic animals. Age-related slowing in movement speed in the elevated plus-maze and open-field tests was comparable in all treatment groups.

Depression-like behavior can be assessed using the Porsolt forced-swim and sucrose preference tests. Our data indicated reduced immobility in rats with TBI. We observed no abnormalities in the consumption of sucrose over water. The epileptic and nonepileptic animals did not differ in any of the outcome measures, consistent with data from Shultz et al.\textsuperscript{14}

5 | CONCLUSIONS

Bröer and Löscher\textsuperscript{25} were able to predict epileptogenesis with an AUC of 0.959 in a rat lithium-pilocarpine model of status epilepticus by combining a threshold for pentylentetrazol-induced seizures, post-pentylenetetrazol behavior, and scores in the pick-up test. Pascente et al.\textsuperscript{26} reported that a slow rate of learning on day 15 and accelerated forgetting on day 65 in Morris water maze were prognostic/diagnostic biomarkers for epileptogenesis after pilocarpine-induced SE with AUCs of 0.790 and 0.840, respectively. In humans, depression is reportedly associated with the evolution of drug-refractory epilepsy.\textsuperscript{44,45} Here we show that outcome parameters in somatomotor, anxiety, depression-like behavior, and spatial memory tests assessed during epileptogenesis did not reveal any sensitive and specific biomarkers for predicting the evolution of PTE. Instead, a simple follow-up of weight decline during the postinjury week appeared as a candidate marker to stratify the risk for PTE after lateral FPI. Whether weight loss is a surrogate marker for the occurrence of post-TBI nonconvulsive SE, thereby predisposing to future epileptogenesis, remains to be studied.

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CONFLICT OF INTERESTS

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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REFERENCES

1. Menon DK, Schwab K, Wright DW, Maas AI. Position statement: definition of traumatic brain injury. Arch Phys Med Rehabil. 2010;91(11):1637–40.
2. Vaishnawi S, Rao V, Fann JR. Neuropsychiatric problems after traumatic brain injury: Unraveling the silent epidemic. Psychosomatics. 2009;50(3):198–205.
3. Juengst SB, Wagner AK, Ritter AC, Szafierski JP, Walker WC, Zafonte RD, et al. Post-traumatic epilepsy associations with mental health outcomes in the first two years after moderate to severe TBI: A TBI Model Systems analysis. Epilepsy Behav. 2017;73:240–6.
4. Hesdorffer DC, Logroscino G, Cascino G, Annegers JF, Hauser WA. Risk of unprovoked seizure after acute symptomatic seizure: effect of status epilepticus. Ann Neurol. 1998;44(6):908–12.
5. Englander J, Bushnik T, Duong T, Cifu DX, Zafonte R, Wright J, et al. Analyzing risk factors for late posttraumatic seizures: A prospective, multicenter investigation. Arch Phys Med Rehabil. 2003;84(3 Suppl. 1):365–73.
6. Herman ST. Epilepsy after brain insult: targeting epileptogenesis. Neurology. 2002;59:S21–S26.
7. Pitkänen A, Engel J. Past and present definitions of epileptogenesis and its biomarkers. Neurotherapeutics. 2014;11(2):231–41.
8. Pittau F, Mégevand P, Sheybani L, Abela E, Grouiller F, Spinelli L, et al. Mapping epileptic activity: Sources or networks for the clinicians? Front Neurol. 2014;5:1–21.
9. Kalitzin S, Petkov G, Suckczynski P, Grigorovsky V, Bardakjian BL, Lopes da Silva F, et al. Epilepsy as a manifestation of a multistate network of oscillatory systems. Neurobiol Dis. 2019;130:104488.

10. Laufs H. Functional imaging of seizures and epilepsy: Evolution from zones to networks. Curr Opin Neurol. 2012;25(2):194–200.

11. Engel J, Pitkänen A. Biomarkers for epileptogenesis and its treatment. Neuropharmacology. 2020;167:107735.

12. Pitkänen A, McIntosh TK. Animal models of post-traumatic epilepsy. J Neurotrauma. 2006;23(2):241–61.

13. Kharatishvili I, Nissinen JP, McIntosh TK, Pitkänen A. A model of posttraumatic epilepsy induced by lateral fluid-percussion brain injury in rats. Neuroscience. 2006;140(2):685–97.

14. Shultz SR, Cardamone L, Liu YR, Hogan RE, Maccotta L, Wright DK, et al. Can structural or functional changes following traumatic brain injury in the rat predict epileptic outcome? Epilepsia. 2013;54(7):1240–50.

15. Campbell JN, Gandhi A, Singh B, Singh B. Traumatic brain injury causes a Tacrolimus-Sensitive increase in non-convulsive seizures in a rat model of post-traumatic epilepsy. Int J Neurol Brain Disord. 2014;1(1):1–11.

16. Reid AY, Bragin A, Giza CC, Staba RJ, Engel J. The progression of electrophysiological abnormalities during epileptogenesis after experimental traumatic brain injury. Epilepsia. 2016;57(10):1558–67.

17. Wang X, Wang Y, Zhang C, Liu C, Yang H-F, Hu W-H, et al. Endogenous cannabinoid system alterations and their role in epileptogenesis after brain injury in rat. Epilepsy Res. 2016;128:35–42.

18. Nissinen J, Andrade P, Natunen T, Hiltunen M, Malm T, Kanninen K, et al. Disease-modifying effect of atipamezole in a model of post-traumatic epilepsy. Epilepsia. 2017;136:18–34.

19. Smith DH, Okiyama K, Thomas MJ, Clausen B, McIntosh TK. Evaluation of memory dysfunction following experimental brain injury using the Morris water maze. J Neurotrauma. 1991;8(4):259–69.

20. Lim SW, Sung KC, Shue YL, Wang C-C, Chio C-C, Kuo J-R. Hyperbaric oxygen effects on depression-like behavior and neuroinflammation in traumatic brain injury rats. World Neurosurg. 2017;100:128–37.

21. Jones NC, Cardamone L, Williams JP, Salzberg MR, Myers D, O’Brien TJ. Experimental traumatic brain injury induces a pervasive hyperanxietous phenotype in rats. J Neurotrauma. 2008;25(11):1367–74.

22. Kuo JR, Cheng YH, Chen YS, Chio C-C, Gao PW. Involvement of extracellular signal regulated kinases in traumatic brain injury-induced depression in rodents. J Neurotrauma. 2013;30(14):1223–31.

23. Immonen RJ, Kharatishvili I, Niskanen J-P, Gröhn H, Pitkänen A, Gröhn OHJ. Distinct MRI pattern in lesional and perilesional area after traumatic brain injury in rat–11 months follow-up. Exp Neurol. 2009;215(1):29–40.

24. Pitkänen A, Ekolle Ndode-Ekane X, Lapinlampi N, Lapinlampi N, Puhakka N. Epilepsy biomarkers – Toward etiology and pathology specificity. Neurobiol Dis. 2019;123:42–58.

25. Bröer S, Löscher W. Novel combinations of phenotypic biomarkers predict development of epilepsy in the lithium-pilocarpine model of temporal lobe epilepsy in rats. Epilepsy Behav. 2015;53:98–107.

26. Pascente R, Frigerio F, Rizzi M, Porcu L, Boido M, Davids J, et al. Cognitive deficits and brain myo-Inositol are early biomarkers of epileptogenesis in a rat model of epilepsy. Neurobiol Dis. 2016;93:146–55.

27. Harris PA, Taylor R, Thielleke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)–a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform. 2009;42(2):377–81.

28. Lapinlampi N, Melin E, Aronica E, Bankstahl JP, Becker A, Bernard C, et al. Common data elements and data management: Remedy to cure underpowered preclinical studies. Epilepsy Res. 2017;129:87–90.

29. Cutpointr TC. Determine and evaluate optimal cutpoints in binary classification tasks. 2019.

30. Andrade P, Paananen T, Ciszek R, Lapinlampi N, Pitkänen A. Algorithm for automatic detection of spontaneous seizures in rats with post-traumatic epilepsy. J Neurosci Methods. 2018;307:37–45.

31. Manninen EM, Chary K, Lapinlampi N, Andrade P, Paananen T, Sierra A, et al. Early increase in cortical T2 relaxation is a prognostic biomarker for the evolution of severe cortical damage, but not for epileptogenesis, after experimental traumatic brain injury. J Neurotrauma. 2020. https://doi.org/10.1089/neu.2019.6796.

32. Dixon CE, Lyeth BG, Povlishock JT, Findling RL, Hamm RJ, Marmarou A, et al. A fluid percussion model of experimental brain injury in the rat. J Neurosurg. 1987;67(1):110–9.

33. McIntosh TK, Vink R, Noble L, Yamakami I, Fernyak S, Soares H, Faden AI. Traumatic brain injury in the rat: characterization of a lateral fluid-percussion model. Neuroscience. 1989;28(1):233–44.

34. Andrade P, Banzuelos-Cabreria I, Lapinlampi N, Paananen T, Ciszek R, Ndode-Ekane XE, et al. Acute non-convulsive status epilepticus after experimental traumatic brain injury in rats. J Neurotrauma. 2019;36(11):1890–907.

35. Bao F, Shultz SR, Hepburn JD, Omana V, Weaver LC, Cain DP, et al. A CD11d monoclonal antibody treatment reduces tissue injury and improves neurological outcome after fluid perfusion brain injury in rats. J Neurotrauma. 2012;29(14):2375–92.

36. Shultz SR, MacFabe DF, Foley KA, Taylor Roy, Cain DP. A single mild fluid perfusion injury induces short-term behavioral and neuropathological changes in the Long-Evans rat: Support for an animal model of concussion. Behav Brain Res. 2011;224(2):326–35.

37. Ferreira VMM, Morato GS. Influence of age and of pre-treatment with D-cycloserine on the behavior of ethanol-treated rats tested in the elevated plus-maze apparatus. Addict Biol. 1996;1(4):395–404.

38. Pietrelli A, Lopez-Costa J, Gitli R, Brusco A, Basso N. Aerobic exercise prevents age-dependent cognitive decline and reduces anxiety-related behaviors in middle-aged and old rats. Neuroscience. 2012;202:252–66.

39. Kim H, Yu T, Cam-Etoz B, van Groen T, Hubbard WJ, Chaudry IH. Treatment of traumatic brain injury with 17α-ethinylestradiol-3-sulfate in a rat model. J Neurosurg. 2017;127(1):23–31.

40. Fucich EA, Mayeux JP, McGinn MA, Gipin NW, Edwards S, Molina PE. A novel role for the endocannabinoid system in ameliorating motivation for alcohol drinking and negative behavioral affect after traumatic brain injury in rats. J Neurotrauma. 2019;36(11):1847–55.

41. Thompson HJ, Lifshitz J, Marklund N, Grady MS, Graham DI, et al. A CD11d monoclonal antibody treatment reduces tissue injury and improves neurological outcome after fluid perfusion brain injury in rats. J Neurosurg. 2005;22(1):42–75.

42. Manschot SM, Biessels GJ, Cameron NE, Cotter MA, Kamal A, Kappelle L, et al. Angiotensin converting enzyme inhibition partially prevents deficits in water maze performance, hippocampal synaptic plasticity and cerebral blood flow in streptozotocin-diabetic rats. Brain Res. 2003;966(2):274–82.

43. Hayward NM, Immonen R, Tuunanen PI, Ndode-Ekane XE, Gröhn O, Pitkänen A, et al. Association of chronic vascular changes with post-traumatic epilepsy induced by lateral fluid-percussion brain injury. Epilepsia. 2013;54(7):1240–50.
with functional outcome after traumatic brain injury in rats. J Neurotrauma. 2010;27(12):2203–19.

44. Kanner AM. Epilepsy, suicidal behaviour, and depression: Do they share common pathogenic mechanisms? Lancet Neurol. 2006;5(2):107–8.

45. Hitiris N, Mohanraj R, Norrie J, Sills GJ, Brodie MJ. Predictors of pharmaco-resistant epilepsy. Epilepsy Res. 2007;75(2–3):192–6.

46. Casillas-Espinosa PM, Andrade P, Santana-Gomez C, Paananen T, Smith G, Ali I, Ciszek R, et al. Harmonization of the pipeline for seizure detection to phenotype post-traumatic epilepsy in a preclinical multicenter study on post-traumatic epileptogenesis. Epilepsy Res. 2019;156:106131.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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