Progression from frontal–parietal to mesial–temporal epilepsy after fluid percussion injury in the rat

Raimondo D’Ambrosio,1,2,4 Jason S. Fender,1 Jared P. Fairbanks,1 Ednea A. Simon,2 Donald E. Born,3,4 Dana L. Doyle3 and John W. Miller1,2

1Department of Neurological Surgery, 2Department of Neurology and Regional Epilepsy Center, 3Department of Pathology and 4Center on Human Development and Disability, University of Washington, School of Medicine, Harborview Medical Center, Seattle, WA, USA

Correspondence to: Raimondo D’Ambrosio, PhD, Harborview Medical Center, Department of Neurosurgery, Box 359915, 325 Ninth Avenue, Seattle, WA 98104, USA E-mail: raid@u.washington.edu

Summary
We recently described an in vivo model of post-traumatic epilepsy (PTE) in the rat where chronic spontaneous recurrent seizures appear following a single episode of fluid percussion injury (FPI). PTE, studied during the first 2 months post-injury, was focal and seizures originated predominantly from the frontal–parietal neocortex at or around the injury site. However, rarer bilateral seizures originating from a different and undefined focus were also observed. To shed light on the posttraumatic epileptogenic mechanisms and on the generation of bilateral seizures, we studied rats up to 7 months post-injury. In vivo paired epidural and depth-electrode recordings indicated that the anterior hippocampus evolves into an epileptic focus which initiates bilateral seizures. The rate of frontal–parietal seizures remained constant over time after 2 weeks post-injury, while the rate of hippocampal seizures greatly increased over time, suggesting that different mechanisms mediate neocortical and hippocampal post-traumatic epileptogenesis. Because of different temporal evolution of these foci, the epileptic syndrome was characterized by predominant frontal–parietal seizures early after injury, but by predominant mesial-temporal seizures at later time points. Pathological analysis demonstrated progressive hippocampal and temporal cortex pathology that paralleled the increase in frequency and duration of bilateral seizures. These results demonstrate that FPI-induced frontal–parietal epilepsy (FPE) progresses to mesial–temporal lobe epilepsy (MTLE) with dual pathology. These observations establish numerous similarities between FPI-induced and human PTE and further validate it as a clinically relevant model of PTE.

Keywords: trauma; drug screening; electrocorticography; partial seizures; gliosis

Abbreviations: FPE = frontal–parietal epilepsy; FPI = fluid percussion injury; GFAP = glial acidic fibrillary protein; MTLE = mesial–temporal lobe epilepsy; PTE = post-traumatic epilepsy; rpFPI = rostral parasagittal fluid percussion injury


Introduction
Traumatic brain injury is one of the most important causes of acquired epilepsy in Western societies (Annegers et al., 1996, 1998) and often manifests itself as complex partial seizures, which are often resistant to treatment with antiepileptic drugs (Mattson et al., 1985; Juul-Jensen, 1986). To compound this problem, no known treatment or agent decreases the progression to epilepsy (Temkin et al., 2001). The hippocampus is at the centre of post-traumatic epilepsy (PTE) research, and different potentially epileptogenic mechanisms have been proposed, including acute hilar neuron loss (Lowenstein et al., 1992), intrinsic membrane depolarization of hilar neurons (Ross and Soltesz, 2000), axonal sprouting (Salin et al., 1995; Golarai et al., 2001; Santhakumar et al., 2001), saturation of synaptic long-term potentiation of Schaffer collaterals (D’Ambrosio et al., 1998), and impaired K+ buffering by post-traumatic glia (D’Ambrosio et al., 1999). However, the clinical significance of these mechanisms in the onset of post-traumatic partial seizures and their
pharmacoresistance has yet to be defined (D’Ambrosio, 2003, 2004). Furthermore, while the hippocampus has a role in human PTE and temporal lobe epilepsy (TLE), as indicated by control of seizures enjoyed by a subgroup of patients following amygda-lopapecancpctectomy (Mathern et al., 1994; Marks et al., 1995; Arruda et al., 1996), head injury patients often develop neocortical foci, suffer from progress-ive temporal cortex pathology and may not be seizure free after hippocampectomy (Marks et al., 1995; Arruda et al., 1996; Diaz-Arrastia et al., 2000). Therefore, understanding the mechanisms by which neocortical and subcortical epilep-tic foci arise, interact and progress following traumatic brain injury is crucial for the development of novel treatments. We recently have developed a model of PTE in the rat where chronic spontaneous recurrent seizures follow a single event of a clinically relevant model of closed head injury, fluid percussion injury (FPI; D’Ambrosio et al., 2003). This model represents a significant departure from previous models of acquired epilepsy because the initiating insult, a transient compression of the dura mater without penetration, is mechan-ically very similar to human cases of closed head injury (McIntosh et al., 1989; Schmidt and Grady, 1993; Schneider et al., 2002). Our previous work demonstrated that, in the first 2 months after injury, FPI induces a predominant epileptic focus in the frontal–parietal neocortex at the injury site, while another epileptic focus, possibly subcortical, was responsible for ~4% of the seizures, suggesting that the FPI-based model of PTE had the neuropathophysiological depth needed to reproduce the complex pathophysiology of the human PTE brain. In the present study, we addressed the following questions: (i) how does the epileptic condition evolve follow- ing the first 2 months post-injury?; (ii) where is the second epileptic focus located?; and (iii) does the activity of these two epileptic foci change over time? We show that multiple epileptic foci develop following FPI and their different temporal evolution results in the progression of frontal–parietal epilepsy (FPE) into mesial–temporal lobe epilepsy (MTLE) with dual pathology.

Material and methods

Animals

Fifty-six outbred male CD Sprague–Dawley rats (colony H-41, Charles River, Hollister, CA), post-natal days 33–35, were used for this study (36 FPI, 20 sham). All procedures were approved by the University of Washington Animal Care and Use Committee.

Rostral parasagittal FPI

Rostral parasagittal FPI (rpFPI) was performed as previously described (D’Ambrosio et al., 2003). Rats were anaesthetized with halothane, intubated and mounted on a stereotaxic frame with the incisor bar adjusted to set bregma 1.5 mm below lambda. A burr hole of 3 mm in diameter was drilled 2 mm posterior to bregma and 3 mm lateral to the midline, on the right convexity. A 10 ms pressure pulse of 3.25–3.5 atm was delivered through the FPI device (Scientific Instruments, University of Washington). After a 10 s pause in breathing upon injury, the animal was re-connected to the ventilator. Shams underwent the same procedure but the pressure pulse was generated with the stopcock of the FPI device closed. The righting time of FPI animals was 12.2 ± 0.7 min (mean ± SEM), while sham rats righted within seconds. The mortality rate by post-traumatic complications was 11%.

Electrophysiology

Montage A consisted of five epidural electrodes, stainless steel screws of $\phi = 1.4$ mm drilled down to the dura to prevent any breach rhythm artefact (Kelly, 2004): a reference electrode placed midline anteriorly over the frontal sinus, and two electrodes per parietal bone at coordinates bregma 0 mm and $-6.5$ mm, 4 mm lateral from the midline. Montage B utilized, in addition, two depth electrodes (Teflon-insulated stainless steel wire, $\phi = 280 \mu$m) vertically lowered at bregma $-3.5$ mm (3.5 mm lateral; vdA) and at bregma $-6$ mm (4.5 mm lateral; vdP) to sample the activity of the ipsilateral anterior and posterior hippocampal CA3 subregion. Montage C was similar, but incorporated only one depth electrode lowered at a 45° angle from bregma $-7.25$ mm (3.5 mm lateral) to sample the activity of the ipsilateral anterior hippocampal CA3 subregion at bregma $-3.5$ mm (ddA) (Fig. 1). The correct position of the depth electrodes was verified by pathology in all animals used in the study. Montage A was implanted 1 week post-injury. In pilot experiments, we observed a drop in hippocampal firing acutely after depth electrode implantation, which subsided in the ensuing days; therefore, montages B and C were implanted 6–18 days before recordings. The electrodes’ headset, video-electrococtygraphy (video-ECOg) acquisition, amplification and storage were as previously described (D’Ambrosio et al., 2003). Electrophysiological data were acquired and analysed with SciWorks with Experimente V2 software (Datawave Technologies Inc., Longmont, CO) and DT3010 acquisition boards (Data-Translation Inc., Marlboro, MA). Eight hours of recordings were performed per rat (sham and FPI) per week. For the study of duration and frequency of seizure, at least 24 h of recordings were performed per rat per time point.

Seizure assessment

Seizure assessment and artefact management were performed with off-line analysis of video-ECOg or depth electrode recordings as previously described (D’Ambrosio et al., 2003). PTE ECOG events were categorized as grade 1 if appearing to be limited to an originating focus (Fig. 2A). Grade 2 activity appeared to originate from a focus and then spread (Fig. 2B). Grade 3 events appeared bilateral at onset (Fig. 2C). In addition to PTE, we observed a total of 37 idiopathic seizures in the whole FPI rat population during this study. They all occurred at 27–28 weeks post-FPI (~3.6% of all cortical...
Animals showing a minimum of two seizures were considered epileptic. Headsets occasionally were lost; when possible, they were re-implanted, otherwise animals were included in the study only until the last day of recording. Epileptic ECoG events occurring within 5 s of each other were defined as belonging to the same seizure event. Behavioural seizure severity was assessed off-line by ranking the behaviour concurrent to epileptiform ECoG events according to the following scale: 0 = no behavioural change (subclinical), 1 = freeze-like pause in behaviour without impaired posture, 2 = freeze-like pause in behaviour without impaired posture and accompanied by facial automatisms (twitching of vibrissae, sniffing, eye blinking or jaw

Fig. 2 Different types of chronic recurrent seizures as revealed by ECoG following FPI. Inset top, left: schematic of the locations of the five cortical electrodes (●) and of the injury site (○) with respect to the rat skull. All panels represent continuous ECoG monitoring at 7 months post-FPI in awake animals. (A) A representative grade 1 post-traumatic seizure detected exclusively by the peri-lesion electrode. (B) A representative grade 2 post-traumatic seizure first detected by the peri-lesion electrode and then by multiple channels. (C) A representative grade 3 post-traumatic seizure detected simultaneously by multiple channels. (D) A representative idiopathic seizure, detected bilaterally and characterized by larger occipital epileptic discharge. These idiopathic seizures were absent until 5.5 months of age and represented 3.6% of all cortical discharge of FPI animals at 8 months of age. ECoG calibration bars are on the left of each panel. Dotted boxes highlight the portion of ECoG shown at higher temporal resolution in the rectangle underneath each panel. The numbers next to each ECoG trace indicate the electrodes by which the trace is recorded, and its reference.
Histology
Histological analyses of brains were performed as previously described (D’Ambrosio et al., 2003). Animals were deeply anaesthetized and perfused transcardially with 4% paraformaldehyde in phosphate-buffered saline (PBS). Brains were removed, post-fixed and cryoprotected in sucrose in phosphate buffer.

GFAP immunoreactivity
Free floating sections (30 μm) were treated to block non-specific staining and incubated with rabbit anti-glia fibrillary acidic protein (GFAP) antibody (1 : 4000 dilution; Dako). Secondary antibody treatment included overnight incubation in a 1 : 300 solution of biotinylated goat anti-rabbit immunoglobulin G (IgG). Biotin–avidin–horseradish peroxidase complexes were formed by incubation in a 1 : 500 Elite ABC kit (Vector Labs, Burlingame, CA). Sections were then developed in 3,3-diaminobenzidine (DAB) and H2O2, mounted on glass slides, air dried, dehydrated through alcohols, cleared in xylene and coverslipped. At least six coronal sections were examined per animal.

Cresyl violet staining
Sections (30 μm) were mounted on glass slides, air-dried, defatted in xylene, stained in cresyl violet solution, differentiated in 95% ethanol, dehydrated through graded alcohols, cleared in xylene and coverslipped.

Morphological analysis
Hippocampal and temporal cortex asymmetry was assessed in 2 × micrographs of cresyl violet-stained coronal sections obtained from bregma −4 mm through bregma −5 mm. Hippocampal area, perimeter, maximum and minimum axes, and temporal neocortex area and perimeter were measured using Object Image 2.10 (http://simon.bio.uva.nl). Duplicate measurements were averaged to reduce variability. The morphological measurements of the hippocampus ipsilateral to the injury were then divided by those of the contralateral hippocampus and multiplied by 100 to obtain a ‘symmetry percentage’. For the temporal neocortex, morphology was assessed by first computing the ratio of each cortical area and its perimeter, which is proportional to the thickness of the neocortex, and then by computing the ipsilateral versus contralateral ratio. In perfect coronal sections from symmetrical brains, all morphological parameters would have a symmetry percentage of 100%. Symmetry percentage decreases as the ipsilateral hippocampus and temporal neocortex shrink due to tissue loss. The highest percentage deviation from 100% among the different morphological parameters examined in the hippocampus and neocortex was taken as their asymmetry. Asymmetry of sham-operated animals ranged between 0 and 7%, and this variability was therefore not considered a pathological sign. A scale was constructed defining asymmetry as ‘negligible’ for asymmetry ≤7%, ‘mild’ if >7% and ≤15%, ‘moderate’ if >15% and ≤30%, and ‘pronounced’ if >30%.

Statistical analysis
All data are presented as mean ± SEM. Statistical comparisons were performed with SPSS 12.0 (SPSS Inc., Chicago, IL). All tests were performed two-tailed, except for the progression of pathology. A P < 0.05 was considered significant.

Results
Probability of developing PTE
Video-ECoG monitoring based on montage A was performed in 24 rpFPI and 20 sham animals for a total of 1882 and 784 h, respectively. Montage A allowed for the detection of three different types of late post-traumatic seizures. Grade 1 and 2 seizures invariably were first detected by the perilesional electrode (Fig. 2A and B), while grade 3 seizures appeared, at the best of our spatial–temporal resolution, bilateral in their cortical onset (Fig. 2C). The cumulative probability that rpFPI rats developed epilepsy reached 100% at 9 weeks post-injury (Fig. 3A). Age-matched shams were recorded at the same time points after surgery for a comparable number of hours and showed no epileptiform ECoG events up to 5.5 months of age (4.5 months post-surgery), consistent with our previous report (D’Ambrosio et al., 2003). In this study, we extended the temporal analysis and found that ~33% of control animals manifested recurrent non-convulsive idiopathic generalized epilepsy at 7–8 months of age. These events, typically 2–10 s long and bilateral in onset, were characterized by a sharp-wave pattern. They were easily distinguished from post-traumatic grade 3 seizures because they were significantly larger in amplitude in the parietal–occipital cortex (Fig. 2D), and were therefore disregarded. At 27–28 weeks post-injury, these idiopathic seizures represented just 3.6% of all cortical discharges.

The cumulative probability that FPI rats developed each grade of post-traumatic seizures was also computed (Fig. 3B). The probability of developing grade 1 seizures increased over time post-injury in a manner identical to the time course of the epileptic condition itself (Fig. 3B), and could be well fit with a single exponential with half-time τ1 = 1.1 weeks. The probability of developing grade 2 or 3 seizures was lower at all times and fit a single exponential with half time τ2 = 3.0 and τ3 = 2.8 weeks, respectively. This suggests that different mechanisms mediate the genesis of grade 1 versus grade 2 and 3 seizures.

Remission from PTE
During these ECoG studies based on montage A, we identified an animal that presented six grade 1 seizures 13 days after injury, but no abnormal ECoG activity thereafter. We considered this animal a case of remission from PTE; it was examined for pathology, but was not included in the studies of seizure frequency and duration.

Temporal evolution of electrical and behavioural seizures
We previously determined that grade 2 electrical seizures increase in proportion over the first 2 months post-FPI,
while grade 3 electrical seizures do not (D’Ambrosio et al., 2003). To determine if FPI-induced PTE continues evolving at later time points after injury, we examined the time dependence of (i) the proportion of each electrical seizure grade; and (ii) the behavioural seizure severity. The first electrophysiological parameter was examined in eight epileptic animals that were recorded weekly from 2 to 28 weeks post-injury (Fig. 4A). At 2–3 weeks post-injury, grade 1 seizures accounted for 91 ± 6.4% of all seizures. This proportion decreased progressively over time, reaching 7.8 ± 6.1% at 27–28 weeks post-injury (P < 0.001). At 2–3 weeks post-injury, grade 2 seizures were only 8.3 ± 5.4% and increased over time, peaking at 36 ± 9.1% at 14–15 weeks post-injury (P = 0.026). Grade 3 seizures were also rare at 2–3 weeks post-injury, being only 5.3 ± 3.1%, and increased over time to 54 ± 11 and 70 ± 13% at 14–15 and 27–28 weeks post-injury, respectively (P < 0.001 versus 2–3 weeks; statistics with paired t test).

Ictal behaviour was examined in the same eight animals. The most common behavioural correlates of electrical seizures during the early weeks post-FPI were stereotyped freeze-like pauses, that were sometimes followed by facial automatisms, and during which the animal did not lose body posture. However, a different behavioural manifestation of electrical seizures became increasingly common over time post-injury. The animal would suddenly interrupt its normal exploratory or grooming behaviour, crawl on the bottom of the cage and stop with its head propped on its forelimbs, staying motionless for 1–10 s, after which grade 3 electrical seizures and sometimes facial automatisms or dystonic posturing of the left hindlimb would appear. We interpret this electro-clinical syndrome as being a complex partial seizure not initiating in the frontal–parietal neocortex and starting during the phase of crawling. We also observed rarer cases of ictal atonia, during which an animal engaged in normal grooming behaviour would suddenly fall head-down to the bottom of the cage, remain motionless for several seconds, and after which righting would be hindered by prolonged atonia of the forelimbs. These events were also consistent with partial seizures. At 7 months post-injury, three animals exhibited prolonged (>30 min) sequences of electrical seizures during abnormally quiet behaviour and without ECoG sleep activity. These events were similar to human non-convulsive partial status epilepticus. The behavioural seizure severity associated with electrical seizures was 1.1 ± 0.2 (range 0–4) at 2–3 weeks post-injury. It increased to 3.1 ± 0.7 (range 1–6) at 17–18 weeks post-injury (P = 0.04) and to 4.1 ± 1.0 (range 1–8) at 27–28 weeks post-injury (P = 0.02; with Mann–Whitney U test versus 2–3 weeks).

Localization of epileptic foci initiating different seizure types

We previously proposed that grade 1 and grade 2 seizures originated from the frontal–parietal neocortex, while a second epileptic focus was responsible for grade 3 seizures (D’Ambrosio et al., 2003). To better determine the location of this second focus, we acquired 336 h of paired epidural and depth electrode video-recordings using montages B and C in six animals at 2–4 weeks post-FPI (192 h) and in another six animals 6.5–7 months post-FPI (144 h). We classified all epileptic events by the location of the first detected epileptic
Fig. 4 Temporal evolution of the FPI-induced post-traumatic epileptic syndrome. Electrical and behavioural correlates of PTE progression are assessed in eight epileptic animals. (A) The proportions of ECoG events of grade 1, 2 and 3 are plotted over time from 2 to 28 weeks post-injury. Focal frontal–parietal seizures (grade 1; ■) represented the most common seizure type 2–3 weeks post-injury, but then progressively decreased in proportion over time. Focal neocortical spreading seizures (grade 2; □) were rare at 2–3 weeks post-injury, but then increased in proportion over time and peaked at 14–15 weeks post-injury. Seizures that did not originate from the frontal–parietal cortex and appeared bilateral in onset (grade 3; △) were rare up to 8–9 weeks post-injury, and then increased up to 27–28 weeks post-injury. Note the overall increase in bilateral spread of the seizures over time post-injury (grades 2 and 3 combined). (B) The behavioural score during epileptiform ECoG events is plotted versus time post-injury. Note the progression of the severity of behavioural seizures over time post-injury. Data are presented as mean ± SEM. Statistics with paired t test (*P < 0.05; **P < 0.01; ***P < 0.001, versus 2–3 weeks).

Table 1 Independent firing and interaction of the frontal–parietal neocortical and hippocampal epileptic foci as observed by paired epidural and depth electrode recordings 6.5–7 months post-FPI

<table>
<thead>
<tr>
<th>Type of occurrence</th>
<th>Proportion</th>
<th>Evidence for focus in</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 1 seizures in the absence of hippocampal discharge</td>
<td>66.7%</td>
<td>Frontal–parietal cortex</td>
</tr>
<tr>
<td>Grade 1 seizures leading to hippocampal discharge</td>
<td>13.9%</td>
<td>Frontal–parietal cortex</td>
</tr>
<tr>
<td>Grade 1 seizures simultaneous with hippocampal discharge</td>
<td>19.4%</td>
<td>Undefined</td>
</tr>
<tr>
<td>Grade 1 seizures preceded by hippocampal damage</td>
<td>0.0%</td>
<td>Not hippocampus</td>
</tr>
<tr>
<td>Grade 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 2 seizures in the absence of hippocampal discharge</td>
<td>2.2%</td>
<td>Frontal–parietal cortex</td>
</tr>
<tr>
<td>Grade 2 seizures leading to hippocampal discharge</td>
<td>80.4%</td>
<td>Frontal–parietal cortex</td>
</tr>
<tr>
<td>Grade 2 seizures simultaneous with hippocampal discharge</td>
<td>17.4%</td>
<td>Undefined</td>
</tr>
<tr>
<td>Grade 2 seizures preceded by hippocampal discharge</td>
<td>0.0%</td>
<td>Not hippocampus</td>
</tr>
<tr>
<td>Grade 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 3 seizures in the absence of hippocampal discharge</td>
<td>0.0%</td>
<td>Not frontal–parietal cortex</td>
</tr>
<tr>
<td>Grade 3 seizures leading to hippocampal discharge</td>
<td>0.0%</td>
<td>Not frontal–parietal cortex</td>
</tr>
<tr>
<td>Grade 3 seizures simultaneous with hippocampal discharge</td>
<td>56.3%</td>
<td>Undefined</td>
</tr>
<tr>
<td>Grade 3 seizures preceded by hippocampal discharge</td>
<td>43.7%</td>
<td>Hippocampus</td>
</tr>
<tr>
<td>Hippocampal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior hippocampus in absence of posterior hippocampus</td>
<td>71.0%</td>
<td>Anterior hippocampus</td>
</tr>
<tr>
<td>Anterior hippocampus leading posterior hippocampus</td>
<td>7.9%</td>
<td>Anterior hippocampus</td>
</tr>
<tr>
<td>Anterior hippocampus simultaneous with posterior hippocampus</td>
<td>21.1%</td>
<td>Undefined</td>
</tr>
<tr>
<td>Anterior hippocampus preceded by posterior hippocampus</td>
<td>0.0%</td>
<td>Not posterior hippocampus</td>
</tr>
<tr>
<td>Interaction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal–parietal seizures in the absence of hippocampal discharge</td>
<td>14.6%</td>
<td>24.4%</td>
</tr>
<tr>
<td>Frontal–parietal seizures leading to hippocampal discharge</td>
<td>16.1%</td>
<td>26.9%</td>
</tr>
<tr>
<td>Hippocampal seizures in the absence of cortical discharge</td>
<td>1.8%</td>
<td>3.0%</td>
</tr>
<tr>
<td>Hippocampal seizures recruiting only frontal–parietal cortex</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Hippocampal seizures recruiting neocortex:</td>
<td>27.4%</td>
<td>45.7%</td>
</tr>
<tr>
<td>Events simultaneous in cortex and hippocampus</td>
<td>40.1%</td>
<td>–</td>
</tr>
</tbody>
</table>

The relative proportion of cortical and hippocampal involvement is shown for each type of seizure (grades 1, 2, 3 and hippocampal). Interaction refers to the reciprocal recruitment of neocortex and hippocampus and it is computed by combining all seizure types and including (w/s.e.) or not (w/o s.e.) events with simultaneous hippocampal and cortical discharge.
activity. In the late group (Table 1), we observed discharge of the frontal–parietal neocortex in the absence of abnormal hippocampal activity (Fig. 5A and C), and of the hippocampus in the absence of abnormal neocortical activity (Fig. 5F), demonstrating that independent neocortical and hippocampal epileptic foci co-exist in the post-FPI epileptic brain chronically after injury. In addition, grade 1 and 2 seizures, always first detected in the frontal–parietal cortex,

![Fig. 5](image-url)

**Fig. 5** Independent firing and cross-talk of the frontal–parietal neocortical and antero-hippocampal epileptic foci. Paired epidural and depth electrode recordings performed in six epileptic animals 6.5–7 months post-injury. (A) A grade 1 seizure detected by the peri-lesion epidural electrode does not recruit the hippocampus, indicating the existence and independent firing of a focus in the frontal–parietal neocortex. (B) Epileptic activity is first detected by the peri-lesion epidural electrode during a grade 1 seizure, and then in the anterior hippocampus, indicating that the neocortical focus recruited the hippocampus. (C) The hippocampus shows no epileptiform activity during the occurrence of a grade 2 seizure that originated around the injury site and then propagated to the frontal contralateral cortex. (D) Epileptic activity is first detected by the peri-lesional epidural electrode in the frontal–parietal cortex, and then spreads ipsi- and contralaterally, during a grade 2 seizure, and to the anterior hippocampus, indicating that the neocortical focus recruited the hippocampus. (E) Epileptiform activity is first detected in the anterior hippocampus and then simultaneously in the ipsi- and contralateral neocortex, indicating that grade 3 seizures originate in the hippocampus. (F) Epileptiform activity is detected only in the hippocampus, in the absence of any neocortical discharge, indicating the existence and independent firing of an epileptic focus in the hippocampus. Scale bars apply to all four traces in each panel. Epileptic bursts shown in E and F are cut off at ±500 µV by gain saturation. Dotted lines and black arrows indicate the beginning of epileptiform activity. Grey arrows indicate propagated epileptic activity. vdA = vertical depth electrode placed in the anterior hippocampus; vdp = vertical depth electrode placed in the posterior hippocampus; dDA = diagonal depth electrode placed in the anterior hippocampus. The text to the left of each ECoG trace indicates the electrode by which the trace is recorded and its reference.
sometimes recruited the hippocampal focus (Fig 5B and D). Grade 3 seizures were always detected in the presence of either leading (Fig. 5E) or simultaneous hippocampal discharge (not shown), but were never observed to precede it, indicating that grade 3 seizures are never initiated by the frontal–parietal focus, but often by the hippocampus. Epileptiform activity detected in the hippocampus was mostly first detected in the anterior hippocampus (Fig. 5F), with or without trailing discharge of posterior hippocampus (Fig. 5E).

Temporal changes in cortical discharge rate and duration

We examined the time course of the rate and duration of cortical discharge as detected by montage A. The total cortical discharge rate, as computed as the count per hour, in each epileptic animals, of all seizure grades, was $2.1 \pm 0.51$ events/h (nine rats; range 0.05–4.4 events/h) at 2–4 weeks and progressively increased to $6.0 \pm 1.8$ (eight rats; range 0.22–16.4 events/h) by 27–28 weeks post-injury ($P = 0.044$; **)

Fig. 6 Temporal evolution of rate of occurrence and duration of cortical and hippocampal discharge. Cortical and hippocampal discharge rate of occurrence (A–D) and duration (E–H) are shown from 2 to 28 weeks post-injury. Cortical discharge occurring during all seizure grades (1, 2 and 3) increased in frequency over time (A). Grade 1 seizures decreased, while grade 2 seizures increased in frequency over time (B). The rate of seizures originating from the frontal–parietal neocortex (grade 1 and 2 combined) did not change from 2–4 weeks to 27–28 weeks post-injury, while the rate of seizures not originating from that focus (grade 3) dramatically increased over time post-injury (C). The frequency of hippocampal seizures increased over time post-injury. Because seizures detected simultaneously in both hippocampus and cortex may also be originating from the hippocampus, the frequency of hippocampal + undefined seizures is shown (D). Cortical discharge duration (all seizure grades) increased over time post-injury (E). The duration of grade 1 seizures did not increase significantly over time, while that of grade 2 seizures did (F). The duration of seizures originating from the frontal–parietal focus (grades 1 and 2) increased, as did the duration of grade 3 seizures that do not originate from it (G). We did not detect a significant increase over time post-injury in the duration of hippocampal discharge during hippocampal seizures. However, the duration of hippocampal + undefined seizures increased significantly over time post-injury (H). Data are presented as mean ± SEM. Statistical comparisons were performed with Wilcoxon signed rank test (A–C) and with Mann–Whitney U test (D–H) versus the 2–4 week time point (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).
The rate of grade 1 seizures decreased from 1.79 ± 0.17 events/h at 2–4 weeks to 0.22 ± 0.1 events/h at 17–18 weeks post-injury (P = 0.008), and persisted at 0.20 ± 0.095 events/hour at 27–28 weeks post-injury (P = 0.018; Fig. 6B). The rate of grade 2 seizures rose from 0.23 ± 0.10 events/h at 2–4 weeks to 1.24 ± 0.78 events/h at 17–18 weeks (P = 0.025) and 2.4 ± 1.29 events/h at 27–28 weeks post-injury (P = 0.12; Fig. 6B). Because grade 1 and 2 electrical seizures appear to originate from the same neocortical epileptic focus, we also evaluated their combined rate as an assessment of the overall activity of that neocortical focus. The combined frontal–parietal seizure rate was 2.04 ± 0.49 events/h at 2–4 weeks and did not change significantly for the duration of the study, being 2.05 ± 0.36 events/h at 17–18 weeks (P = 0.68) and 2.6 ± 0.53 events/h at 27–28 weeks post-injury (P = 0.24; Fig. 6C). Conversely, the rate of grade 3 seizure progressively increased from 0.06 ± 0.027 events/h at 2–4 weeks to 3.7 ± 0.76 at 27–28 weeks post-injury (P = 0.017; Fig. 6C; statistics with Wilcoxon signed rank test versus 2–4 weeks).

The duration of all cortical discharges combined was 4.8 ± 0.30 s (range 0.5–57 s) at 2–4 weeks post-injury (n = 389), that increased to 8.3 ± 0.43 s (range 0.5–89 s) at 17–18 weeks (n = 470; P < 0.001), and to 40 ± 0.78 s (1–88 s) at 27–28 weeks post-injury (n = 280; P < 0.001; Fig. 6E). Grade 1 seizures lasted 4.2 ± 0.26 s at 2–4 weeks post-injury (n = 322), and persisted at 2.84 ± 0.50 s at 17–18 weeks (n = 29; P = 0.11), and at 4.17 ± 0.97 s at 27–28 weeks post-injury (n = 9; P = 0.42). Grade 2 seizures lasted 8.7 ± 1.3 s at 2–4 weeks post-injury (n = 55), persisted at 9.13 ± 0.71 s at 17–18 weeks (n = 162; P = 0.13), and increased to 12.7 ± 1.5 s at 27–28 weeks post-injury (n = 101; P = 0.02; Fig. 6F). The duration of frontal–parietal seizures (grades 1 and 2 combined) was 4.84 ± 0.31 s at 2–4 weeks (n = 377), and increased to 8.17 ± 0.63 s at 17–18 weeks (n = 191; P < 0.001), and to 12.0 ± 1.4 s at 27–28 weeks post-injury (n = 110; P < 0.001; Fig. 6G). Similarly, grade 3 seizures lasted 2.46 ± 0.44 s at 2–4 weeks post-injury (n = 12), and increased in duration to 8.43 ± 0.58 s at 17–18 weeks (n = 279; P < 0.001), and to 11.79 ± 0.89 s at 27–28 weeks post-injury (n = 170; P < 0.001; Fig. 6G; all statistics with Mann–Whitney U test versus 2–4 weeks).

**Temporal changes in hippocampal discharge rate and duration**

We examined the occurrence of hippocampal discharge as detected by montages B and C. The number of animals showing hippocampal seizures, as defined as those in which the hippocampus fired first or alone, was ~33% (two out of six) at 2–4 weeks post-FPI, and increased to 100% (six out of six) at 26–27 weeks post-injury, demonstrating progressive MTLE. We then examined the temporal changes in rate and duration of hippocampal discharge as detected by 312 h of recording from epileptic animals with montages B and C. The rate of hippocampal seizures was 0.033 ± 0.02 events/h (5 rats) at 2–4 weeks and increased to 1.24 ± 0.60 events/h (six rats) at 26–27 weeks (Fig. 6D; P = 0.004). Seizures appearing simultaneously in hippocampus and cortex (Table 1) have undefined origin, but some or all of these may originate from the hippocampus. Their inclusion brought our estimate of the hippocampal seizure rate to 0.1 ± 0.05 events/h at 2–4 weeks and to 2.75 ± 1.18 events/h at 26–27 weeks (Fig. 6D; P = 0.004, both statistics with Mann–Whitney U test).

The duration of hippocampal discharge during hippocampal seizures was 5.9 ± 1.3 s (n = 8; range 2–10 s) at 2–4 weeks and 6.8 ± 0.36 s (n = 147; range 2–28 s) at 26–27 weeks (Fig. 6H; P = 0.55). The inclusion of seizures appearing simultaneously in hippocampus and cortex brought our estimate of the duration of hippocampal discharge to 4.1 ± 0.7 s (n = 20) at 2–4 weeks and to 6.6 ± 0.24 s (n = 339) at 26–27 weeks (Fig. 6D; P < 0.001, both statistics with Mann–Whitney U test).

**Structural substrates of the progression of PTE**

We examined time-dependent changes in brain pathology in 21 FPI and eight sham animals. Coronal sections obtained from the early group (2–4 weeks post-FPI) and stained for cresyl violet showed remarkable neuronal loss and calcifications in the ipsilateral thalamus. The ipsilateral hippocampus and temporal neocortex presented either no or mild shrinkage without loss of laminar features (not shown). GFAP immunoreactivity was markedly increased in the ipsilateral thalamus, hippocampus and frontal–parietal cortex in all FPI animals, while a focus of glial reactivity was apparent in the temporal cortex of epileptic animals (D’Ambrosio et al., 2003). Coronal sections obtained from the late group (27–28 weeks post-FPI) and stained for cresyl violet showed remarkable neuronal depletion and calcifications in the thalamus of all FPI animals (Fig. 7A and B). The ipsilateral hippocampus (Fig. 7B2) and temporal neocortex (Fig. 7B4) presented varying degrees of shrinkage among animals, ranging from negligible to pronounced with loss of laminar features (Fig. 7B4). Atrophic hippocampi were characterized by atrophy of CA1 and CA3 subfields (Fig. 7B1 and 2). Numerous small nuclei, presumably of glial cells, were observed in ipsilateral hippocampus and temporal cortex (Fig. 7B4, inset). We quantitatively assessed the temporal changes in asymmetry of hippocampus and temporal neocortex. In the early group, FPI animals, epileptics and non-epileptics, presented either no (~43%) or mild (~57%) hippocampal asymmetry (Fig. 7C, left panel), and either no (~43%) or mild (~57%) asymmetry in the temporal cortex (Fig. 7D, left panel). In the late group, FPI animals presented varying degrees of hippocampal asymmetry (Fig. 7C, right panel), ranging from negligible (~14%), mild (~36%), moderate (~36%) to pronounced (~14%), as well as a different degree of temporal cortex asymmetry (Fig. 7D, right panel), ranging from negligible (~21%), mild (~29%), moderate (~29%) to pronounced (~21%). The differences in hippocampal and temporal cortex asymmetry between the early and late time points were statistically significant (P = 0.02 and P < 0.04, respectively; one-tailed Mann–Whitney...
Fig. 7 Progressive hippocampal and temporal cortex pathology in the post-traumatic epileptic rat. Coronal sections obtained from bregma −4 mm through bregma −5 mm and stained for cresyl violet and GFAP. (A) GFAP immunoreactivity of an epileptic animal 7 months post-injury demonstrating no hippocampal and mild temporal cortex asymmetry. At higher magnification, cresyl violet staining shows symmetric contralateral (A1) and ipsilateral (A2) hippocampi and no loss of laminar features. (B) GFAP immunoreactivity of another epileptic animal 7 months post-injury demonstrating pronounced hippocampal and temporal cortex asymmetry. At higher magnification, cresyl violet staining shows asymmetric contralateral (B1) and ipsilateral (B2) hippocampi, with evident atrophy of ipsilateral CA3 and CA1 subregions. The contralateral temporal cortex showed no atrophy (B3a) and normal laminar structure (B3b). However, the ipsilateral temporal cortex was atrophic (B4a) and showed pronounced loss of neurons and laminar features, and increased small nuclei representing reactive glia (B4b), all typically associated with temporal cortex sclerosis. Hippocampal (C) and temporal cortex (D) asymmetry increased over time post-injury in the population of FPI animals. Statistics with one-tailed Mann–Whitney U test. FPI rem. = case of PTE remission following FPI; FPI epi. = persistently epileptic animals; FPI n.epi. = not epileptic animals. Scale bars: 1 mm for A and B, 250 μm for A1–2 and B1–B4b. Black arrows in A and B indicate temporal foci of glial reactivity. Dotted circles in A and B delineate the thalamic injury present in all rpFPI animals. Dotted rectangles in B3a and B4a delineate the areas magnified in B3b and B4b, respectively.
U test). The degree of temporal cortex asymmetry correlated with the degree of hippocampal asymmetry ($R = +0.76; P < 0.005$).

**Discussion**

The main finding of the present study is that a single episode of rpFPI is sufficient to induce independent cortical and hippocampal epileptic foci whose activity evolves differently over time post-injury, resulting in changes in the epileptic syndrome and a progressive development of MTLE.

**rpFPI-induced PTE is a progressive disorder**

rpFPI-induced PTE manifests itself at the cortical level with three different types of electrical seizures in the first weeks post-injury (D’Ambrosio et al., 2003). We have now followed their evolution up to 7 months post-injury and found that their relative proportion, in each epileptic animal, changes dramatically over time. Grade 1 seizures, detected only in the neocortex at or around the injury site (Fig. 2A), are the most common type in the first 2 months after injury (Fig. 3B), but rapidly drop in proportion to represent only ~5% of all seizures by 7 months post-injury (Fig. 4A). While it is possible that the thalamic injury found in all rpFPI animals may generate an epileptic thalamo-cortical loop, the findings of intrinsically hyperexcitable frontal–parietal cortex in slices in vitro and of non-epileptic (D’Ambrosio et al., 2003) and remission cases observed in spite of comparable thalamic injury suggest that the epileptic focus responsible for grade 1 seizures may lie within the frontal–parietal cortex itself. Grade 2 seizures spread from the injury site and represent secondarily generalized focal events. They progressively increased in proportion over the first 2 months post-injury (D’Ambrosio et al., 2003), indicating that focal seizures spread more easily over time. We now show that grade 2 seizures peak in proportion at ~14–15 weeks post-injury (Fig. 4A). The cellular substrates of their increase are likely to be different from those responsible for the onset of grade 1 seizures and may include progression of pathology and/or kindling of cortico-cortical and cortico-subcortical pathways (Goddard, 1967). Consistent with this hypothesis, epileptogenesis responsible for grade 2 seizures had a half-time longer than that of grade 1 seizures (Fig. 3B). Grade 3 seizures, which are bilateral at their cortical onset (Fig. 2C), are rare in the first 2 months post-injury, representing only ~5% of the overall seizure activity, but dramatically increased in proportion over the following months, reaching ~65% of all cortical discharge by 7 months post-injury (Fig. 4A). Their simultaneous bilateral appearance demonstrates that the frontal–parietal neocortex is not the focus generating them. Therefore, they probably propagate to the neocortex via subcortical pathways. Indeed, paired epidural and depth electrode recordings indicate they can originate from the hippocampus.

In addition to their relative proportion, seizures also changed in rate of occurrence and duration over the months post-injury. While grade 1 seizures decreased, grade 2 seizures increased in frequency over time (Fig. 6B). We interpret their opposite and complementary evolution as due to their common origin in the frontal–parietal focus, as indicated by our previous work (D’Ambrosio et al., 2003) and by paired epidural–depth electrode recordings (Fig. 5; Table 1). We surmise that the worsening of grade 1 seizures results in grade 2 seizures. We therefore can represent the overall activity of the frontal–parietal focus as the sum of the rates of grade 1 and 2 seizures. This activity was constant over the months post-FPI (Fig. 6C), suggesting that the frontal–parietal focus develops within 2 weeks post-injury and that the epileptogenic mechanisms responsible for its onset do not significantly affect its firing rate after this critical period. However, pro-epileptic mechanisms continue to work after this temporal window and result in the increase in duration of both frontal–parietal (grade 1 and 2 combined) and grade 3 seizures (Fig. 6G). Contrary to frontal–parietal seizures, cortical discharge during grade 3 seizures also increased in rate of occurrence (Fig. 6C) over the months post-injury. We have indication that 40–60% of grade 3 seizures originate in the anterior ipsilateral hippocampus and are therefore hippocampal seizures (Fig. 5E; Table 1). Indeed, we found that the firing rate of the anterior hippocampus (Fig. 6D) dramatically increases over time post-injury. The remainder of grade 3 seizures may originate from either a different area of the hippocampus, possibly the contralateral one, or from a third epileptic focus (see below). The dramatic time-dependent increase in hippocampal firing rate may be due to kindling induced by the relentless activity of the frontal–parietal focus. Alternatively, it may be an epiphenomenon accompanying the underlying progression in brain pathology. Further experiments are needed to elucidate the issue and the different mechanisms responsible for seizure onset, maintenance and spread in the frontal–parietal neocortex and hippocampus.

The progression of FPI-induced epilepsy was also evident at the behavioural level and further demonstrated the evolution from FPE to TLE. At 2–3 weeks post-injury, the behavioural correlate of electrical seizures was typically a freeze-like pause in behaviour, with or without facial automatisms, consistent with an epileptic focus located in the forebrain (Browning, 1986, 1987). However, at 5–7 months post-injury, animals typically displayed stereotyped electro-clinical events consisting of a sudden interruption in behaviour, followed by crawling, and then by trains of grade 3 electrical seizures during which motor manifestations, typically facial automatisms and contralateral hindlimb dystonia, often occurred. Both of these behavioural seizures are similar to complex partial seizures in humans; the former sometimes observed when the focus is in the frontal cortex (Williamson and Spencer, 1986) and the latter typically observed in TLE (Kuba et al., 2003). We also observed rarer events of ictal atonia during which animals engaged in grooming behaviour would fall and remain unresponsive and motionless for several seconds. These events are similar to atonia occurring during partial seizures as observed in patients with either
frontal epilepsy or FPE (Tinuper et al., 1998; Satow et al., 2002). We did not observe tonic–clonic convulsions, as expected due to the employment of rpFPI which does not result in significant damage to the brainstem, a structure involved in their onset (Browning, 1987; Gale and Browning, 1988). In addition, most of our rats were recorded only 8 h/week, and sufficiently rare tonic–clonic convulsions may have been missed. However, more caudal FPI is known to result in greater damage to the motor cortex, hippocampus and brainstem and should result in more frequent tonic–clonic seizures. Further experiments are needed to assess the relationship between FPI site and epileptic syndrome.

**Cross-talk between neocortical and hippocampal epileptic foci**

In addition to the independent firing of the frontal–parietal and hippocampal epileptic foci, numerous cases of their interaction were observed (Table 1). The frontal–parietal cortex was capable of rather selectively recruiting the anterior hippocampus (Fig. 5B and D), but selective recruitment of the frontal–parietal cortex by the hippocampus was never observed. Hippocampal activity first detected in the hippocampus (Fig. 5B and D), but selective recruitment of the hippocampus by a frontal–parietal cortex pathology following rpFPI that manifests itself as predominant ipsilateral atrophy and loss of laminar features (Fig. 7). While at 2–4 weeks post-injury, 57% of the animals have significant hippocampal and temporal cortex asymmetry, by 7 months post-FPI hippocampal and temporal cortex asymmetry becomes significant in 86 and 79% of the cases, respectively. Our choice to perform rpFPI is instrumental in this observation because a more posterior FPI causes greater acute damage to the hippocampus and temporal cortex (Cortez et al., 1989; Floyd et al., 2002) which hinders the observation of a later progressive sclerosis, typical of MTLE, by confounding it with acute and subacute post-traumatic neuronal loss. While the temporal cortex pathology progresses to a clear sclerosis, as defined by significant loss of neurons and lamina features with increased gliosis, in 79% of the animals we did not observe loss of laminar features in the hippocampus. However, Grady et al. (2003), employing an identical rpFPI and stereological cell count, demonstrated 50% neuronal loss in the ipsilateral hilus and 23% in the ipsilateral CA3 subregion 2 weeks post-injury. Therefore, neuronal loss occurred and the progressive hippocampal atrophy we observed is likely to be an early stage of progressive sclerosis. Indeed, human hippocampal sclerosis is a progressive disorder (Fuerst et al., 2003), and hippocampi studied are typically resected from MTLE patients 17–18 years after diagnosis (Salanova et al., 2002; Benbadis et al., 2003), and probably after several more years of subclinical seizures, therefore representing extremely chronic cases. In agreement with this view, atrophy in rpFPI animals was mostly evident in CA1 and CA3 subfields, as is expected in the earlier stages of human hippocampal sclerosis. Further experiments are needed to determine the cellular bases of this progressive hippocampal atrophy. Interestingly, the observed heterogeneity in hippocampal and temporal cortex pathology defines different subpopulations of rpFPI epileptic animals, just like different subpopulations of human epileptic patients suffering from TLE exist with different degrees of hippocampal or temporal cortex atrophy, cognitive or mnemonic disturbances, and pharmacoresistant complex partial seizures (French et al., 1993; Mathern et al., 1995; Fuerst et al., 2003). We surmise that differences in the epileptic condition of the animal, and therefore in seizure-induced hippocampal kindling and neuronal loss (Sloviter, 1983; Cavazos and Sutula, 1990; Holmes, 2002), and differences in genetic background (Schauwecker, 2002; McKhann et al., 2003), may all account
for the different progression of hippocampal and temporal cortex pathology.

**A case of remission from PTE**

During this study, we identified a case of remission from PTE. At the pathological level, the brain showed mild hippocampal asymmetry and pronounced injury to the ipsilateral thalamus (not shown), which are therefore entirely attributable to the acute injury and not to the epileptic condition. As expected, this animal did not present a focus of glial reactivity in the temporal cortex, as all other epileptic animals in this study did, and as previously reported (D’Ambrosio et al., 2003). Cases of remission also occur in human PTE (Frey, 2003), and the finding of similar cases in the FPI rat population increases the numerous similarities found between this rodent model and the human condition (Table 2).

**Cases of age-dependent idiopathic epilepsy in the colony**

Sprague-Dawley rats purchased from Charles Rivers’ colony H41, and used for this and our previous study (D’Ambrosio et al., 2003), did not exhibit idiopathic seizures within their first 5.5 months of life, but we found cases at later age. About 33% of the animals presented bilateral posterior epileptic ECoG events, associated with no obvious brain pathology at 7–8 months of age, that were likely to be a manifestation of age-dependent idiopathic epilepsy. These idiopathic seizures presented with a pattern of sharp waves that was similar to that seen in many other laboratory rat strains (Van Luijftelaar and Coenen, 1986; Inoue et al., 1990; Willoughby and Mackenzie, 1992; Vadasz et al., 1995), but it was different from true spike–wave discharge (a term often used for the different progression of hippocampal and temporal cortex pathology.

**Table 2 Similarities between rpFPI-induced PTE in the rat and human PTE**

<table>
<thead>
<tr>
<th>Case</th>
<th>Description</th>
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<tbody>
<tr>
<td>1.</td>
<td>The traumatic brain injury model is mechanically very similar to human cases of closed head injury.</td>
</tr>
<tr>
<td>2.</td>
<td>Chronic spontaneous recurrent seizures appear after a single event of traumatic brain injury.</td>
</tr>
<tr>
<td>3.</td>
<td>Seizure free ‘latent period’ between the initiating injury and the onset of the epileptic condition.</td>
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<tr>
<td>4.</td>
<td>Seizures are focal, with or without secondary bilateral spread.</td>
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<tr>
<td>5.</td>
<td>The ictal behaviour is consistent with human complex partial seizures.</td>
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<tr>
<td>7.</td>
<td>Preferential recruitment of hippocampus by frontal–parietal foci, but not vice versa.</td>
</tr>
<tr>
<td>8.</td>
<td>Progressive temporal lobe sclerosis in a subgroup of epileptic individuals, and not in others.</td>
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<tr>
<td>9.</td>
<td>Time-dependent changes in epileptic syndrome.</td>
</tr>
<tr>
<td>10.</td>
<td>Cases of remission.</td>
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rpFPI-induced PTE in the rat reproduces many of the features of human PTE and shows faster epileptogenesis in the neocortex at the injury site than in the mesial temporal lobe, resulting in temporal evolution from FPE to dual pathology with MTLE. The temporal changes in type and frequency of neocortical and hippocampal partial seizures observed offer the unprecedented opportunity to study different aspects of clinically relevant post-traumatic epileptogenesis and to examine the drug sensitivity of partial seizures that represent the real obstacle in the treatment of pharmacoresistant epilepsy.

**Conclusions**

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