Post-traumatic epilepsy following fluid percussion injury in the rat

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Summary
The lack of an adequate model of post-traumatic epilepsy (PTE), in which, similarly to the human condition, chronic spontaneous focal seizures follow a single episode of traumatic brain injury, has hampered the identification of clinically relevant epileptogenic mechanisms and the development of effective therapies. We studied the electrophysiological, behavioural and structural consequences of a clinically relevant model of closed head injury, the lateral fluid percussion injury (FPI), in the rat. We found that a single episode of severe FPI is sufficient to cause PTE. Chronic electrocorticography (ECoG) demonstrated spontaneous chronic seizures that were partial, originated from the neocortex at the site of injury, and progressively worsened and spread over time. The cases of epilepsy in the post-traumatic population increased over time following injury. Post-FPI epileptic rats exhibited pauses in their behaviour, facial automatisms and myoclonus at the time of epileptiform ECoG events. In vitro local field potential recordings demonstrated persistent hyperexcitability of the neocortex at and around the site of injury that was associated with intense glial reactivity. These results for the first time demonstrate persistent hyperexcitability of the injured neocortex and define a useful model for pathophysiological studies of basic mechanisms of spontaneous epileptogenesis and for preclinical screening of effective antiepileptogenic drugs.

Keywords: traumatic brain injury; epileptogenesis; electrocorticography; drug screening; gliosis.

Abbreviations: BBB = blood–brain barrier; ECoG = electrocorticography; FPI = fluid percussion injury; GFAP = glial fibrillary acidic protein; LFP = local field potential; PTE = post-traumatic epilepsy; TBI = traumatic brain injury


Introduction
Traumatic brain injury (TBI) is a major risk factor for subsequent development of epilepsy (Feeney and Walker, 1979; Temkin et al., 1996; Annegers et al., 1998), and often results in chronic seizures that are poorly controlled by currently available drugs (Temkin et al., 2001; Lösch and Schmidt, 2002). Unfortunately, no animal model of post-traumatic epilepsy (PTE) reproducing the human post-traumatic condition exists to elucidate the pathophysiological substrates of post-traumatic epileptogenesis and to perform preclinical screening of antiepileptogenic drugs (Schmidt and Rogawski, 2002; Stables et al., 2002; White, 2002). After experimental closed head injury, several laboratories have reported acute seizures (Nilsson et al., 1994), and hippocampal hyperexcitability to stimulation (Lowenstein et al., 1992; Coulter et al., 1996; Santhakumar et al., 2001) or to proconvulsant drug exposure (Coulter et al., 1996; Reeves et al., 1997; Golarai et al., 2001), but spontaneous chronic seizures, the hallmark of epilepsy, have never been described. However, no long-term electrophysiological monitoring of cortical activity in vivo has ever been reported. In addition, while neocortical epileptic foci commonly develop in humans following TBI (Diaz-Arrastia et al., 2000), changes in neocortical excitability have never been studied in rodent models of closed head injury, which have all focused on hippocampus (D’Ambrosio, 2003). Therefore, two approaches are currently left to study neocortical pathophysiology in PTE: the first consists of neocortical islands isolated by undercuts from the surrounding grey matter (Grafstein and Sastry, 1957;
Sharpless and Halpern, 1962; Echlin and Battista, 1963) and the second consists of chronic neocortical implantation of ferrous chloride (Willmore et al., 1978; Willmore and Rubin, 1984). Neocortical islands have been used recently in a series of experiments that elucidated possible PTE mechanisms (Prince and Tseng, 1993; Hoffman et al., 1994; Graber and Prince, 1999). However, both models bear little resemblance to the human post-traumatic condition because they lack its unique focal and diffuse mechanical and haemorrhage components. Therefore, these models are not optimal for identifying the main clinically relevant epileptogenic mechanisms and leading to significant discoveries that can be translated to the human condition.

In the present study, we addressed two crucial questions pertaining to epileptogenesis following TBI. First, is a single episode of lateral fluid percussion injury (FPI), a clinically relevant model of closed head injury, sufficient to cause PTE in the rat? Secondly, what are the electrophysiological and immunohistochemical substrates of PTE? We hypothesized that chronic seizures following a single episode of closed head injury have never been observed because (i) no long-term electrophysiological monitoring of post-traumatic rats has ever been performed; and (ii) closed head injury in the rat causes subtle behavioural partial seizures that have no obvious early motor involvement.

Material and methods
All procedures were approved by the University of Washington Animal Care and Use Committee.

Lateral FPI
FPI was carried out as described previously (D’Ambrosio et al., 1999). Briefly, male Sprague–Dawley rats (post-natal days 32–35) were anaesthetized with halothane and intubated. A burr hole of 3 mm in diameter was drilled 2 mm posterior to the bregma and 3 mm lateral to the midline, on the right convexity. A brief (10 ms) pressure pulse of 3.75–4 atm on the intact dura was delivered while the animal was disconnected from the ventilator. After a 10 s pause in breathing upon injury, the animal was re-connected to the ventilator. The righting time of FPI animals was 18.0±0.9 min (n = 16; mean ± SEM), while sham rats righted within seconds.

Cortical recordings
Five to seven days post-injury, rats were anaesthetized as per FPI, and epidural electrodes were implanted for electrocorticography (ECoG). This procedure resulted in no chronic cortical damage, as assessed by glial fibrillary acidic protein (GFAP) and cresyl violet histology. We used three montages. Montage A consisted of five electrodes: one was placed midline in the frontal bone and used as reference, while two electrodes per parietal bone were placed at coordinates bregma 0 mm and –6.5 mm, 4 mm from the midline. Montage B was similar, but two additional electrodes were placed at coordinates bregma –3.5 mm, 1.5 mm from the midline. Montage C’s features were one additional electrode at the centre of the injury site, another on the right parietal bone, between the temporal ridge and the temporo-mandibular joint at bregma –6.5 mm, and a third on the squamosal bone, midway between the temporal ridge and the temporomandibular joint at bregma –3.5 mm. All electrodes were connected through insulated wire to a gold-plated pin in a plastic pedestal which was cemented onto the skull. ECoG and video monitoring began on day 14 post-FPI. Rats were placed singly in plexiglass cages where they could move freely. Electrical brain activity was amplified (×10 000–20 000), filtered (low-pass 100 Hz cut-off) using a 16-channel amplifier (Neurodata 12, Grass Instruments, Quincy, MA, USA), digitized at 1.6 kHz, stored, and analysed on a Pentium-based computer equipped with Experimenter’s Workbench 5.3 and Datawave acquisition board (Datawave Technologies Inc., Longmont, CO, USA). Behaviour was monitored throughout the recordings and stored on video tape using SuperVHS cameras. Eight hours of recordings were performed per rat per time point.

Seizure assessment
Seizures were assessed with off-line analysis by at least two investigators (R.D., J.S.F. and J.P.F.) who examined ECoG recording and synchronized video-monitoring. A third investigator (J.W.M.) was blinded to the treatment and graded undecided events. ECoG events showing a sudden increase in voltage amplitude, and a >5-fold increase in power of frequencies >10 Hz were marked as possibly epileptic. The behaviour of the animal was studied for each potentially epileptiform ECoG event detected. Rhythmic scratching of the electrode headset by the animal rarely caused artefacts: such artefacts were easily identified and discarded. Stereotyped high-voltage artefacts that coincided with chewing were observed in very few cases in naïve, sham and FPI animals, and were discarded as muscular artefacts. Epileptiform ECoG events were categorized as grade 1 if appearing to originate from a focus, and being limited to it. Grade 2 activity appeared to originate from a focus and then spread. Grade 3 events appeared, at the best of our temporal resolution, simultaneously in multiple channels. Thus, grade 1 and 2 epileptiform events represent partial seizures, while grade 3 events represent secondary generalized seizures. In this study, animals showing seizures every week for a minimum of 3 weeks were considered epileptic thereafter. Animals that did not show seizures were considered to be non-epileptic only until the last day of recordings.

Behavioural seizure severity was assessed off-line by three investigators (R.D., J.S.F. and J.P.F.) by ranking the concurrent behaviour of epileptiform ECoG events according to a modified Racine’s scale (Racine, 1972): 0 = no behavioural change (subclinical), 1 = pause in behaviour, 2 = facial movements (twitching of vibrissae, sniffing, eye blinking or jaw automatisms), 3 = mild head nodding, 3.5 = severe head nodding, 4 = myoclonus. Cases when animals’ behaviour could not be appreciated because of their unfavourable position with respect to the camera, or because they were already motionless, were excluded from analysis.

In vitro electrophysiology
Neocortical slices were obtained from epileptic animals 8–10 weeks post-FPI. Animals were transcardially perfused with ice-cold solution composed of (in mM): 3.1 KCl, 1.25 KH2PO4, 3 MgCl2, 1 CaCl2, 26 NaHCO3, 10 dextrose and 206 sucrose. Rats were decapitated and the heads collected in the same solution supplemented with kynurenic acid (1 mM). A coronal block of tissue was obtained (Bregma +1 to –2.5 mm), glued on the stage of a Vibratome 100Plus, and submerged in fresh ice-cold solution with kynurenic.
acetic acid. Coronal slices (400 μm thick) were obtained and gently transferred to a holding chamber containing an artificial CSF (ACSF) composed of (in mM): 120 NaCl, 3.1 KCl, 1.25 KH2PO4, 2 MgCl2, 2 CaCl2, 26 NaHCO3 and 10 dextrose. Slices were incubated at 35°C for 30 min, and then were kept at room temperature until used. All solutions were equilibrated with 95% O2:5% CO2 to a final pH of 7.35. For recording, slices were gently transferred to a submersion chamber and constantly superfused at a rate of ~1 ml/min with oxygenated ACSF composed of (in mM): 120 NaCl, 3.1 KCl, 1.25 KH2PO4, 1 MgCl2, 2 CaCl2, 26 NaHCO3 and 10 dextrose. All the electrophysiological recordings were carried out at 35°C. Field potentials were recorded from layer V of each cortical slice with extracellular pipettes (3–5 MΩ) filled with equilibrated ACSF.

**Histology**

**GFAP immunoreactivity**

Between week 6 and 16 post-surgery, animals were deeply anaesthetized with pentobarbital and perfused transcardially with 4% paraformaldehyde. Brains were removed, post-fixed, and cryoprotected in sucrose in phosphate buffer. Free floating sections (30 μm) were incubated in a solution of 3% normal goat serum (NGS), 0.3% Triton-X (TX) and 3% bovine serum albumin (BSA) in 0.1 M phosphate-buffered saline (PBS; pH 7.4) for 1 h to block non-specific staining. Sections were treated with primary antisera containing anti-GFAP antibody (1 : 4000 dilution; Dako) in 0.1 M PBS with 1% NGS, 0.3% TX and 1% BSA overnight. A rinse solution of biotinylated goat anti-rabbit immunoglobulin (IgG) in PBS with 1% NGS, 0.3% TX and 1.0% BSA, and then rinsed.

Sections were then mounted on glass slides, air dried, dehydrated through alcohols, cleared in xylene and coverslipped. At least six coronal sections were examined per animal. Qualitative correlation between the presence of chronic electrical seizures in FPI rats and glial reactivity in the temporal cortex ipsilateral to the injury site was performed as follows: coronal neocortical slices, randomly chosen from 15 sham-operated and 11 FPI animals, were independently observed at 40× by three investigators (R.D., J.S.F. and J.P.F.) who were blind to the treatment. Glial reactivity in the temporal cortex was qualitatively rated as ‘absent’, ‘moderate’ and ‘pronounced’.

**Cresyl violet staining**

Sections were mounted on glass slides, air dried, defatted in xylene, stained in cresyl violet solution (0.1% cresyl violet, 0.01% acetic acid), differentiated in 95% ethanol, dehydrated through graded alcohols, cleared in xylene, and coverslipped.

**Results**

Severe TBI was induced in 16 rats, while 18 age-matched shams were used as controls. Severe lateral rostral FPI was chosen to maximize the likelihood of observing chronic seizures because (i) injury severity positively correlates with likelihood of chronic seizures in humans (Annegers et al., 1996, 1998); (ii) lateral FPI produces more cortical damage than central FPI (McIntosh et al., 1989; Schmidt and Grady, 1993); and (iii) during a pilot study, we determined that a rostral FPI cannula allowed us to deliver a severe injury with minimal loss of animals due to acute post-traumatic complications, possibly because the pressure wave shock was directed away from the brainstem.

**Spatial–temporal evolution of chronic post-traumatic seizures**

We performed paired chronic ECoG and video monitoring from 2 to 16 weeks post-injury. Epileptiform activity was readily observed chronically after FPI in ~92% of the animals during their normal wake behaviour (12 out of 13 FPI rats at 10 weeks post-injury). All grade 1 and 2 epileptiform events were first detected by the electrode adjacent to the neocortex at the site of injury (Fig. 1). Grade 2 events were observed to spread secondarily to adjacent areas of the neocortex. Fast Fourier transform (FFT) analysis revealed that the spectral power in the 7–30 Hz range was significantly greater during epileptiform ECoG events relative to baseline. In addition, we found that the spectral power of the ECoG trace immediately after the epileptiform events was significantly less than baseline at 7 Hz, suggesting post-ictal depression (Fig. 1).

One of the features of human PTE is the silent period, between the traumatic event and the onset of chronic seizures, during which epileptogenic mechanisms are thought to take place. To determine whether FPI caused a similar development of epilepsy, we plotted the cumulative probability that rats displayed electrical seizures over time post-injury (Fig. 2). About 60% of the animals were observed to have epileptiform ECoG events 2 weeks post-injury (eight out of 13 animals). The probability of developing epilepsy increased over time and reached 92% at 8 weeks post-injury (11 out of 12 animals). About 32% of the rats were not epileptic 2 weeks following FPI but developed seizures at chronic time points. No sham-operated animal ever showed any epileptiform ECoG events.

To determine if FPI causes partial seizures that worsen over time, we considered the time dependence of (i) the likelihood that epileptiform ECoG activity first detected in the right frontal–parietal neocortex then spreads to other areas; and (ii) the behavioural seizure severity (Fig. 3). The first electrophysiological parameter was examined in five epileptic animals that were recorded from 2 weeks post-injury.
onwards. The number of epileptiform ECoG events of one grade versus the number of all epileptiform ECoG events was plotted against time (Fig. 3A). Grade 1 events were observed most frequently at 2 weeks post-injury, with a proportion $R_1 = 0.92 \pm 0.07$, that progressively decreased to $0.51 \pm 0.13$ at 8 weeks post-injury (mean ± SEM $n = 5$; $P = 0.026$). Conversely, spreading events were rarely observed 2 weeks post-injury, with a proportion $R_2 = 0.05 \pm 0.053$, that
progressively increased to 0.45 ± 0.13 at 8 weeks post-injury \((n = 5; P = 0.026)\). Grade 3 events were rare at 2 weeks post-injury, with a proportion R3 = 0.023 ± 0.023, that remained constant to 0.04 ± 0.022 at 8 weeks post-injury \((n = 5; P = 0.7)\). The decrease in frequency of grade 1 seizures, and the corresponding increase in frequency of grade 2 events, were observed in all animals studied (Fig. 3B). The second behavioural parameter was examined in the same animals. Except in those cases when animals were already motionless, post-FPI epileptic rats showed pauses in their behaviour in synchrony with all three grades of epileptiform ECoG events. Pauses in behaviour are a normal component of the rat’s behavioural repertoire, and were commonly observed during normal ECoG activity in both sham-operated and FPI animals. However, epileptiform ECoG activity observed in post-traumatic rats was nearly invariably associated with paused behaviour that was sometimes followed by facial automatisms, head-nodding or myoclonus. Some of the grade 1 ECoG events at 2 weeks post-injury did not correspond to obvious pauses in behaviour and were therefore considered to be subclinical. The behavioural seizure severity during epileptiform ECoG events, as assessed by a modified Racine’s scale (Fig. 3C), was 0.96 ± 0.07 (range 0–4) at 2 weeks post-injury and increased to 2 ± 0.3 at 8 weeks post-injury (range 1–4; mean ± SEM; \(P = 0.028\)). The increase in behavioural seizure severity was observed in all animals studied (Fig. 3D).

**Localization of chronic neocortical hyperexcitability**

To better assess the neocortical involvement in chronic spontaneous seizures, and their possible pathways of secondary generalization, six epileptic animals were examined 9–16 weeks post-injury (Fig. 4). Montage B (three animals) allowed us to better map the activity of the two hemispheres of the brain. Electrode 4 was kept adjacent to the site of FPI, while electrodes 6 and 7 mapped the somatosensory cortex. This montage confirmed the existence of two types of epileptiform ECoG events: those where epileptiform activity at the region of electrode 4 led (Fig. 4 A, middle panel), and those where epileptiform activity appeared simultaneously in multiple channels (Fig. 4 A, right panel). No seizure ever appeared to originate from fields other than that of electrode 4. Montage C allowed us to better map the activity of the ipsilateral parietal, occipital and temporal cortex. Recordings from three epileptic rats revealed that: (i) epileptiform

![Fig. 2 Probability of unprovoked seizures following severe lateral FPI. The cumulative probability of detecting epileptiform ECoG events in post-FPI (grey bars) and sham-operated (white bars) rats is plotted versus time after injury. Numerators indicate the number of epileptic rats, while denominators indicate the number of animals evaluated in the group.](image)

![Fig. 3 Evolution of chronic seizures following lateral FPI. Electrical and behavioural correlates of PTE progression are assessed in five animals epileptic at week 2 post-injury. (A) The proportions of events of grade 1, 2 and 3 are plotted over time from 2 to 8 weeks post-injury. Focal events (grade 1; filled square) were most frequently observed at 2 weeks post-injury. Spreading events (grade 2; filled circles) increased over time post-injury. Generalized events (grade 3; open triangle) remained constant up to 8 weeks post-injury. (B) The increase in proportion of spreading epileptiform ECoG events was observed in all animal studied. (C) The behavioural score during epileptiform ECoG events is plotted at 2 and 8 weeks post-injury. Subclinical seizures were only observed at 2 weeks, while facial automatisms, head nodding and myoclonus were more frequently observed at 8 weeks post-injury. (D) The increase in behavioural seizure score was observed in all animals studied. Each circle–diamond pair represents one animal evaluated at 2 and 8 weeks, respectively. Data are presented as mean ± SEM. Statistics with paired \(t\) test.](image)
activity appeared to initiate at, and sometimes was limited to, the frontal–parietal cortex at the site of injury sampled by electrodes 4 and 6 (Fig. 4B, middle panel); (ii) no epileptiform activity that initiated in the temporal neocortex could be detected; and (iii) grade 2 epileptiform ECoG events with temporal–neocortical involvement occurred following bursting of the fronto-parietal focus (Fig. 4B, right panel). These data provided additional evidence that the first neocortical manifestation of PTE occurs at or surrounding the site of FPI.

To corroborate these ECoG data, we performed local field potential (LFPs) recordings in acute coronal slices of the neocortex obtained from bregma 0 mm/±1 mm (Fig. 5). Slices were obtained from the left and right hemispheres of four epileptic FPI rats (10 slices) and two sham-operated rats (five slices), 8–10 weeks post-surgery. Four different subregions were studied in each neocortical slice: frontal, forelimb area, parietal I and parietal II. Each subregion was stimulated and recorded per slice per experimental group. Slices obtained from sham-operated rats responded with typical LFPs in layer V, consisting of an antidromic population spike sometimes followed by a single orthodromic population spike, and never developed afterdischarges or spreading-depression-like events when stimulated for 1–3 min at 0.1 and 1 Hz (no slices out of five; Fig. 5A). Conversely, slices obtained from epileptic FPI rats were often hyperexcitable in one or more subregions of the neocortex. Slices obtained from the right hemisphere (n = 5) of FPI animals displayed afterdischarges when stimulated at 0.1 and 1 Hz for 1–3 min. Abnormal neuronal excitability was elicited from the frontal, forelimb, parietal I and parietal II areas in one, four, two and three out of five of the studied slices, respectively. Slices obtained from the left hemisphere contralateral to the injury site (n = 5) were less prone to afterdischarge and spreading depression. Here, abnormal neuronal activity was seen only in the frontal area in one out of five of the slices tested (Fig. 5B). This experiment demonstrates that the neocortex at or surrounding the site of FPI is intrinsically hyperexcitable at chronic time points.

Structural substrates of PTE

We examined cresyl violet and GFAP staining in nine FPI and eight sham-operated animals. Histopathological changes were predominantly limited to the injured hemisphere, and involved neocortex and thalamus. In coronal sections, obtained 6–16 weeks after FPI and stained with cresyl violet, neuronal depletion was evident in the temporal neocortex (Fig. 6C1) and in the thalamus, where areas of calcification were commonly observed (Fig. 6C2). Neuronal loss was not evident in the neocortex beneath the FPI cannula (Fig. 6A and

**Fig. 4** Frontal–parietal and parietal–temporal ECoG. (A) Representative recording of a spreading partial seizure originating from the frontal–parietal neocortex sampled by electrode 4 (central traces). Note it is first detected by electrode 4, and then by the other electrodes placed both ipsilaterally and contralaterally. A representative recording of a generalized seizure appearing in multiple channels simultaneously, albeit with different amplitudes (right traces). (B) Representative recording of a partial seizure limited to the frontal–parietal neocortex (middle traces). From the amplitude of the epileptic burst, the focus can be estimated to be approximately at the site of FPI. Representative recording of partial seizures that originated at the site of FPI and were then propagated to the temporal cortex (right traces). Insets, left: schematic of the location of the seven cortical electrodes (filled circles) and of the injury site (open circle). The montage is indicated next to each ECoG trace. ECoG calibration bars are on the left. In all traces, vertical dotted lines mark the first detectable epileptiform activity.
B), but numerous small nuclei, presumably of glial cells, were observed, in particular at the site of injury (Fig. 6B1), in the parietal (Fig. 6B2) and in the temporal cortex (Fig. 6C1). No significant neuronal depletion was apparent in the contralateral neocortex. Alternate sections obtained from the same animals were stained for GFAP immunoreactivity. We were interested in post-traumatic changes in the neocortical glia because reactive gliosis is a prominent feature of human PTE, and because post-traumatic reactive astrocytes have been found to undergo electrophysiological changes that may contribute to synchronous neuronal activity in the rat hippocampus (D’Ambrosio et al., 1999, 2002; Schroder et al., 1999). GFAP immunoreactivity was most pronounced in the hemisphere ipsilateral to the injury displayed widespread hyperexcitability when stimulated at 0.1 and 1 Hz for 1–3 min (five slices). The contralateral hemisphere had a lower tendency toward afterdischarge generation (five slices) than the ipsilateral one. Fr = frontal cortex; FL = forelimb area of cortex; Par = parietal cortex, area I; Par II = parietal cortex, area II. Filled circles mark areas where stimulation could elicit afterdischarges or spreading depression. The configuration of stimulating and recording electrodes is displayed representatively at the left forelimb cortex. Artefacts of the stimuli were clipped for clarity. Calibration bars represent 0.5 mV and 20 ms throughout the figure. Note that each pair of LFPs acquired at 0.1–1 Hz has different scale bars.

**Correlation between glial reactivity and the presence of epilepsy**

Because one of the most prominent features of the human PTE brain is marked gliosis, we performed a qualitative test of whether GFAP immunoreactivity in the parieto-temporal cortex correlated with the presence of chronic electrical seizures (Fig. 7). This study was performed blind by examining sections obtained at bregma –3 mm/–6.5 mm. Staining intensity of the cloud of glial reactivity in the parieto-temporal cortex was rated ‘absent’, ‘mild’ or ‘pronounced’. Of eight sham and six FPI animals whose histology was studied 9–16 weeks post-injury, we found that none of the shams had any detectable temporal foci of glial reactivity. Conversely, all FPI animals that were epileptic at the time of histology (five rats) showed a temporal focus of glial reactivity that was pronounced in four and mild in one of them. One FPI animal that was not epileptic (4 months post-injury) did not present a temporal focus of glial reactivity.

**Discussion**

The main findings of the present study are that (i) a single episode of lateral FPI, a clinically relevant model of human closed head injury, is sufficient to cause PTE in the rat; (ii) seizures emerge after a silent period, are partial and worsen over time post-injury; (iii) epileptiform electric activity is first detected in the neocortex at the site of injury, which is chronically hyperexcitable after FPI; and (iv) intense glial...
Fig. 6 Cellular necrosis and reactive glial response after lateral FPI. Cresyl violet staining (A–D) and GFAP immunoreactivity (E–H) in coronal sections from injured animals. From top to bottom, micrographs and inserts of representative regions are shown for stereotaxic coordinates bregma 0 mm, bregma –2 mm, bregma –3.5 mm and bregma –6.5 mm. Chronically following lateral FPI (6–16 weeks post-injury) there is a marked decrease in large cell bodies (neurons), and a marked increase in small cell bodies attributed to marked gliosis in the ipsilateral temporal lobe (C). In addition, at the same time, there is a marked increase in GFAP immunoreactivity of the hemisphere ipsilateral to the injury site. Clouds of GFAP-positive reactive glial cells were observed from the white matter and grey–white matter interface of the right forelimb parietal cortex (E) throughout the cortical layers of the occipital/temporal cortex (G and H). Scale bars are 1 mm and 50 μm for all the low and high magnification plates, respectively.
reactivity is observed in epileptic animals from the site of injury to the temporal cortex. Thus, electrophysiological and structural sequelae of severe lateral FPI in the rat parallel the changes seen in human PTE.

**PTE and chronic neocortical hyperexcitability following FPI**

The severity of brain injury is the most important predictor of PTE in humans, that occurs in ~7% of the patients with severe head injury (Annegers et al., 1998), and in 30% of those whose admission Glasgow Coma Scale rating is <10 (Temkin et al., 1996). In addition, it has been shown that the risk of PTE significantly increases with intracerebral and subdural haematomas, and cortical contusion (Jennett, 1975; Annegers et al., 1996; 1998; Temkin et al., 1996), all features that are well reproduced in the rat brain by lateral FPI (McIntosh et al., 1989; Schmidt and Grady, 1993). At the cellular level, lateral FPI causes hippocampal sclerosis (Hicks et al., 1996) and reactive gliosis (Hill-Felberg et al., 1999) that are features of the human post-traumatic brain. In addition, lateral FPI alters neurotrophin and neurotrophin receptor levels in the hippocampus (Hicks et al., 1999) and reactive oxygen species (Povlishock and Kontos, 1992; Marklund et al., 2001), both of which are thought to contribute to the secondary injury process and to epileptogenesis (Hall, 1989; Juurlink and Paterson, 1998; Binder et al., 2001; Scharfman et al., 2002). Several laboratories have therefore attempted to employ FPI, and similar closed head injury models, to study post-traumatic changes in neuronal excitability and seizure (Lowenstein et al., 1992; Couler et al., 1996; D’Ambrosio et al., 1999; Golarai et al., 2001; Santhakumar et al., 2001). However, it previously has not been observed that a single event of closed head injury is sufficient to cause spontaneous chronic seizures in the rat. This led to the acceptance that a rodent model of PTE based on closed head injury was not possible perhaps because of the low probability of chronic seizures. However, the ECoG and behavioural data presented here demonstrate that spontaneous focal seizures develop chronically after severe lateral FPI, in agreement with PTE in humans (Jennett, 1975). It therefore appears that seizures were not observed previously following FPI because chronic ECoG was not employed, because behavioural assessment was terminated too soon after injury and/or was not aided by ECoG, or because the degree and type of injury were not adequate.

FPI-induced PTE manifested itself with three different types of ECoG events. Grade 1 seizures consist of focal events that were first invariably detected at, or surrounding, the neocortex at the site of injury (Figs 1 and 4). *In vitro* electrophysiology demonstrated that such a neocortical area was prone to seizure precipitation, whereas the homologous contralateral neocortex presented only very modest hyperexcitability (Fig. 5). More studies are required to determine the nature of such chronic focal neocortical hyperexcitability, but its presence supports the existence of a neocortical epileptic focus. Grade 2 seizures consist of focal events that appeared first at or surrounding the neocortex at the site of injury and then spreaded ipsi- and/or contralaterally (Figs 1 and 4). These events, which represent secondarily generalized seizures, progressively increased in proportion over time post-injury, indicating that post-traumatic epilepsy worsened over time. Indeed, behavioural seizure score also increased over time post-injury (Fig. 3). Possible substrates of the worsening of seizures are a progression of neuronal necrosis and/or gliosis observed in neocortical areas distal from the injury site (Fig. 6) and seizure-induced kindling of specific pathways (Goddard, 1967). Grade 3 seizures consist of rare generalized events during which, at the best of our spatial–temporal resolution, epileptiform activity was detected simultaneously in multiple channels (Fig. 4). The existence of such events and the fact that they do not increase in proportion over time suggest that post-FPI animals may suffer from a dual pathology: the neocortex at or surrounding the site of injury may be an epileptic focus, but subcortical structures may be involved in the origin of rarer generalized events. Indeed, limbic structures can precipitate PTE in humans, and further experiments are required to clarify their role in PTE in the rat.

**Histopathology of the post-FPI epileptic rat brain**

It remains to be elucidated why focal seizures are invariably first detected in the neocortex close to the site of injury. One
possibility is that focal blood extravasation is one of the factors necessary for the development of an epileptic focus and, interestingly, it was shown that blood–brain barrier (BBB) breakdown occurs in the neocortex at the site of lateral FPI (Cortez et al., 1989; Schmidt and Grady, 1993). However, lateral FPI also causes widespread breakdown of the BBB in the medulla, thalamus and hippocampus (Cortez et al., 1989; Schmidt and Grady, 1993), and prominent limbic seizures would be expected if BBB breakdown was the predominant epileptogenic factor. Conversely, and in spite of severe histopathology found in the thalamus, but not in the hippocampus (Fig. 6), seizures of possible limbic origin appeared to be 4% (grade 3 seizures). The unremarkable pathology we observed in the hippocampus is probably due to the more rostral lateral injury employed in the present study, while previous work demonstrating significant hippocampal pathology employed a more caudal lateral FPI (Lowenstein et al., 1992), and is in agreement with the analysis of craniectomy position versus brain tissue damage performed by Floyd et al. (2002). Special quantitative techniques will be needed to assess subtle differences in hippocampal pathology following rostral lateral injury.

In the cortex, we observed neuronal loss, chronically following FPI and in agreement with reports by others (Cortez et al., 1989; Schmidt and Grady, 1993; Floyd et al., 2002). A cloud of intense glial GFAP-positive immuno-reactivity reaching from the injury site to the temporal cortex (Fig. 6) was also observed in the neocortex of epileptic animals chronically after injury. Interestingly, this pattern of glial reactivity closely resembles the pattern of acute disruption of vascular permeability following lateral FPI (Cortez et al., 1989; McIntosh et al., 1989). However, one FPI animal remarkably differed from this pattern. In spite of the fact that this animal was severely injured, as assessed by the 10 s interruption of spontaneous breathing immediately after FPI and by its righting time of 21 min, it never showed any epileptiform activity and it did not present chronic (16 weeks) glial reactivity in the ipsilateral temporal neocortex. This exception suggests that chronic glial reactivity following FPI may not be spatially linked to the acute histopathological features but, rather, to the epileptic condition of the animal. The presence of glial reactivity in the temporal cortex in epileptic animals may be induced by epileptiform activity itself (Steward et al., 1991). Alternatively, glial reactivity may be coincidental to inflammatory processes that may exacerbate it (Norenberg, 1994) and promote seizure precipitation at the same time (Vezzani et al., 1999, 2000). Finally, glial reactivity may facilitate seizure precipitation and spread (Rao et al., 1998; D’Ambrosio et al., 1999). Therefore, further experiments are required to determine whether neocortical reactive glia have altered physiology and contribute to PTE. We surmise that a variety of focal changes associated with cortical contusion, ranging from BBB extravasation, inflammation, glial reactivity, neuronal damage and reorganization, cooperate in the generation of an epileptic focus.

Conclusions
In summary, we have developed an in vivo rodent model of PTE that lends itself to both physiological and preclinical studies. The model reproduces many of the features of human PTE and suggests an epileptogenic role for the neocortex at the site of injury. We believe the process of identifying specific epileptogenic mechanisms, and discovering anti-epileptogenic agents, will be advanced by its use.

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