A rapid lateral fluid percussion injury rodent model of traumatic brain injury and post-traumatic epilepsy

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Traumatic brain injury is a leading cause of acquired epilepsy. Initially described in 1989, lateral fluid percussion injury (LFPI) has since become the most extensively used and well-characterized rodent traumatic brain injury and post-traumatic epilepsy model. Universal findings, particularly seizures that reliably develop after an initial latent period, are evident across studies from multiple laboratories. However, the LFPI procedure is a two-stage process, requiring initial surgical attachment of a skull fluid cannula and then reanesthesia for delivery of the epidural fluid pressure wave. We now describe a modification of the original technique, termed 'rapid lateral fluid percussion injury' (rLFPI), which allows for a one-stage procedure and thus shorter operating time and reduced anesthesia exposure. Anesthetized male Long–Evans rats were subjected to rLFPI through a length of plastic tubing fitted with a pipette tip cannula with a 4-mm aperture. The cannula opening was positioned over a craniectomy of slightly smaller diameter and exposed dura such that the edges of the cannula fit tightly when pressed to the skull with a micromanipulator. Fluid percussion was then delivered immediately thereafter, in the same surgery session. rLFPI resulted in nonlethal focal cortical injury in all animals. We previously demonstrated that the rLFPI procedure resulted in post-traumatic seizures and regional gliosis, but had not examined other histopathologic elements. Now, we show apoptotic cell death confined to the perilesional cortex and chronic pathologic changes such as ipsilesional ventriculomegaly that are seen in the classic model. We conclude that the rLFPI method is a viable alternative to classic LFPI, and – being a one-stage procedure – has the advantage of shorter experiment turnaround and reduced exposure to anesthetics. *NeuroReport* 25:532–536 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Traumatic brain injury (TBI) is a prevalent cause of acquired epilepsy [1–3] and results in a variety of early-onset and late-onset pathological changes [4] that likely contribute to chronic, poorly controlled seizures [5,6]. The clinical coupling between TBI and post-traumatic seizures, as well as the absence of adequate antiepileptogenic prophylaxis, drives an active body of translational research reliant on rodent TBI models.

First described over two decades ago [7], lateral fluid percussion injury (LFPI) has become the most extensively used and best-characterized rodent post-traumatic epilepsy (PTE) model. Universal findings, such as apoptotic cell death [8–10], ipsilesional ventriculomegaly [11–13], mild and transient motor deficits [14,15], and spontaneous recurrent post-traumatic seizures which develop 6–8 weeks after injury [16,17], are evident across studies from multiple laboratories, serving to increase the reliability of the LFPI model. These findings reflect neuropathologic processes believed to be involved in post-traumatic epileptogenesis, and as such, this rodent TBI model approximates the human post-traumatic condition and enables the study of the pathophysiology of post-traumatic epileptogenesis and also the screening of antiepileptogenic drugs.

The classic LFPI procedure, however, is an onerous one as it involves two stages: (i) an initial craniotomy followed by the fixing of a Luer-lock over the exposed intact dura using dental cement or cyanoacrylate, and (ii) after the adhesive has dried, connecting the animal to the LFPI apparatus before delivery of a rapid epidural pulse of sterile saline into the cranium and subsequent removal of the Luer-lock. In addition, some groups perform the first stage using injectable anesthetics, allow the animal to recover overnight, and then perform the second stage under inhaled anesthesia [18,19]. Thus, the classic LFPI method is a two-stage procedure requiring long and repeated anesthetic exposures, sometimes over a 24-h

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period. Yet, numerous studies demonstrate the neuroprotective effects of common injectable and inhaled anesthetic agents and narcotic analgesia [20,21], and these may be significant confounding factors when analyzing data obtained from intervention trials using the classic LFPI model.

Here we describe a modification in the original LFPI technique – a one-stage procedure termed 'rapid lateral fluid percussion injury' (rLFPI) – which allows for much shorter operating times and reduced anesthesia and analgesia exposure. Previously, we reported the epileptogenicity of this model in rats, with 100% of animals developing epilepsy after rLFPI [22]. We now demonstrate that essential histopathological and anatomical findings observed after the classic two-stage LFPI procedure are consistently seen after rLFPI as well. As follows, we tested whether fluid percussion delivered through a cannula that is tightly approximated, but not cemented, to the skull immediately after the initial craniectomy will have sequelae that are similar to those in the established two-stage LFPI model, and whether the additional variability has a major effect on the findings.

Materials and methods

Animals

Adult male Long–Evans rats (367 g, SD: 35.8 g; $N = 17$) were used for the present experiment. All animals were housed in a temperature-controlled animal care facility with a 12 h light–dark cycle. All procedures were approved by and in accordance with the guidelines of the Institutional Animal Care and Use Committee at Boston Children's Hospital (Boston, Massachusetts, USA) and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of rats used in the present experiments.

Rapid lateral fluid percussion injury

In contrast to the established LFPI protocol, we performed a rapid one-stage procedure to deliver fluid percussion to exposed dura. Animals ($N = 13$) were anesthetized with 2–4% isoflurane vapor (Baxter Pharmaceutical, Deerfield, Illinois, USA) and placed in a stereotaxic frame. Anesthesia was then maintained through the rest of the surgery session with 1–3% isoflurane. Under sterile conditions, a 2 cm midline incision was made into the scalp, extending anteriorly from the interaural line, and held open using a self-retaining retractor. The periosteum was then cleared from the skull using a periosteal elevator, and the bregma, lambda, and left lateral ridge were identified. Using a trephine saw, a 4 mm craniectomy was performed over the left parietal cortex with the center of the craniectomy 2 mm posterior and 3.5 mm lateral to the bregma for motor injury, and 2 mm anterior and 3.5 mm lateral to the lambda for histology, taking care not to damage the dura, which was carefully examined to confirm its integrity both before and after fluid percussion (Fig. 1a). A 35-cm long polyvinyl chloride tubing was securely fitted to the male connector of the fluid percussion device (AmScien Instruments, Richmond, Virginia, USA) and the connection was made airtight using polytetrafluoroethylene tape. The free end of the tubing was attached to a pipette tip that had been cut at the end, forming a cannula with an aperture with a 5 mm external and 3.5 mm internal diameter, to overlap fully with the margins of the craniectomy (Fig. 1a). The cannula was then placed in a beaker of sterile saline, and the air inside the tubing was completely flushed out by pushing in the plunger at the other end of the LFPI device. The plunger was then slowly pulled back to its original position while the cannula was still in the beaker, priming the tubing with sterile saline and ensuring there were no air bubbles. Two to three drops of sterile saline were also placed over the craniectomy, and the cannula was positioned over the exposed dura using a micromanipulator, such that the edges of the cannula fit tightly on the skull around the craniectomy (Fig. 1b). In contrast to classic LFPI, the cannula was not cemented into place. Rather, the cannula, tubing, and column of sterile saline were maintained over the craniectomy and exposed dura using the stereotaxic micromanipulator.

A percussion wave of 2.15 atm (± 0.08 atm) at the cannula tip was delivered rapidly (22 ms) to induce the injury. The animal's breathing was visually monitored, and the duration of apnea following the delivery of the percussion wave was recorded using a stopwatch to standardize the injury severity and the contribution of immediate post-traumatic hypoxia. The average duration of apnea after delivery of the percussion wave was 9.5 s (SEM: 2.53 s). Animals that experienced apnea for longer than 15 s were excluded from analysis.

Rat body temperature was maintained with a 40°C warming pad until the animals were able to ambulate, at which point they were transferred to their home cages. Rats were administered opioid analgesics for 48 h after rLFPI.

Beam walking task

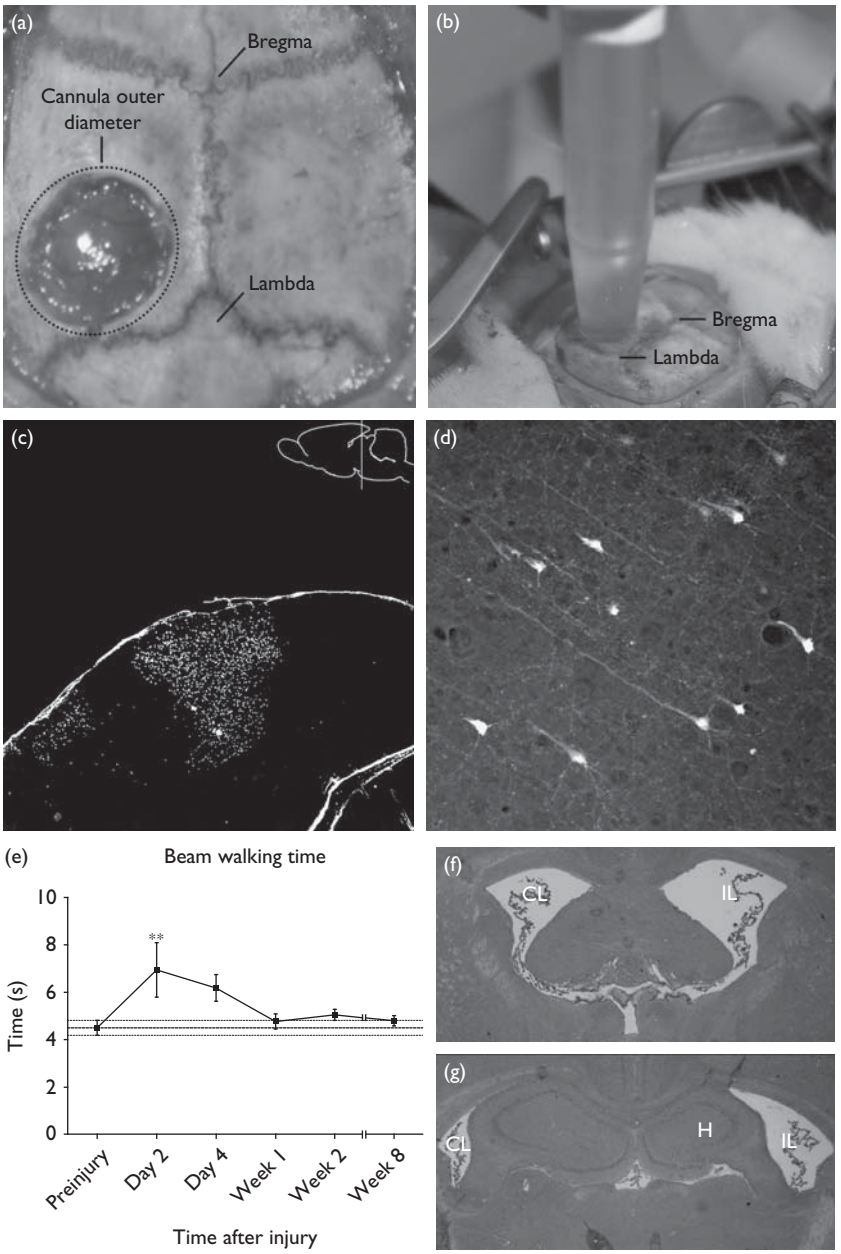
To assess whether our method caused motor dysfunction comparable with the classic LFPI procedure, animals were trained to escape from a bright light toward a dark home cage by traversing a Plexiglas beam (120 cm long, 2.5 cm wide) four times before formal recording. After the fourth practice run, the animals were subjected to five subsequent test runs, which were video recorded, and beam walking time was averaged over these five runs.

Histology

Tissue harvesting and sectioning

Animals were deeply anesthetized with Nembutal (H. Lundbeck A/S, Valby, Denmark) and transcardially perfused with 4% paraformaldehyde in PBS. Brains were carefully removed and fixed in 4% paraformaldehyde at

Fig. 1



Key features of classic LFPI are also seen in rLFPI. (a) Location of craniectomy with intact, exposed dura. Dotted line shows area of bone where cannula wall will overlap. (b) Placement of cannula over craniectomy using micromanipulator. (c) TUNEL shows that rLFPI results in well-demarcated wedge-shaped regions of apoptotic cell death confined to the ipsilesional cortex, 24 h after injury (× 4 magnification). (d) FJB+ degenerating neurons were observed in the ipsilesional cortex and hippocampus 24 h after injury (× 20 magnification). (e) Repeated measures one-way ANOVA indicated significant differences between preinjury and postinjury time [$F_{(5,41)}=3.61$, $P<0.01$]. Post hoc tests showed that beam walking time increased significantly on Day 2 after rLFPI before returning to baseline by 1 week after injury. Error bars indicate SEM. Dotted horizontal line represents mean baseline time, solid lines represent baseline SEM. $^{**}P<0.01$. (f, g) Enlargement of the ipsilesional (IL) lateral ventricle in relation to the contralesional (CL) ventricle was observed 12 weeks after injury, implying ipsilesional hippocampal degeneration (H). ANOVA, analysis of variance; FJB, Fluoro-Jade B; LFPI, lateral fluid percussion injury; rLFPI, rapid lateral fluid percussion injury; TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labeling.

4°C overnight. Fixed brains were submerged in 30% sucrose solution and then rapidly frozen in Tissue-Tek OCT Compound (Sakura Finetek USA Inc., Torrance, California, USA) before being sectioned into 30-μm thick sections using a cryostat. Every tenth section was mounted onto a charged slide.

Terminal deoxynucleotidyl transferase dUTP nick-end labeling

Terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) [23] was performed using the *in situ* cell death detection kit TMR Red (Roche Diagnostics, Indianapolis, Indiana, USA). Briefly, slides were washed in PBS for 30 min and then incubated in permeabilization solution (0.1% Triton X-100, 0.1% sodium citrate, freshly prepared) for 2 min on ice. Slides were rinsed twice with PBS and then incubated in TUNEL reaction mixture for 60 min at 37°C in a darkened, humidified chamber. The slides were then rinsed three times in PBS and cover-slipped using antifade mounting medium. The sections were examined using a fluorescence microscope with an excitation wavelength between 520 and 560 nm and a detection wavelength in the range of 570–620 nm.

Fluoro-Jade B staining

Sections were stained with Fluoro-Jade B (FJB) to quantify neuronal degeneration [24]. Slides were immersed in 100% ethanol for 3 min, followed by 1 min in 70% ethanol, and 1 min in distilled water and were then transferred to a solution of 0.06% potassium permanganate for 15 min, followed by a 1-min rinse in distilled water. The slides were stained with 0.001% FJB solution (Histochem, Jefferson, Arkansas, USA), prepared in 0.1% acetic acid, for 30 min, and then rinsed for 1 min in distilled water three times. They were then dried in a 37°C oven in the dark for over 1 h before being cleared by immersion in xylene and cover-slipped with DPX (Fluka, Milwaukee, Wisconsin, USA). Coronal sections, 250 μm apart, were examined from 1 to 6 mm posterior to the bregma. Two blinded observers rated FJB + cells in the lesional and contralesional cortices and both hippocampi on a 0–3 ordinal scale, in which 0 = no FJB-positive cells, 1 = less than 10% of total neurons, 2 = approximately 10–50% of total neurons; 3 = more than half the cells or fibers are FJB positive in the area of interest, as published by Hallam *et al.* [25]. Both scores were averaged for a structure–location score that was used for all FJB quantification.

Ventricle volume measures

To measure ventriculomegaly, sections taken at 1 mm intervals along the entire length of the lateral ventricles were stained with H&E and imaged at × 2 magnification. The ventricles were outlined and measured using ImageJ software (NIH, Bethesda, Maryland, USA).

Results and discussion

The primary injury caused by classic LFPI is rapid, transient deformation of the brain parenchyma, leading to direct mechanical trauma and necrotic cell death. Thereafter, a complex series of biochemical and molecular events leads to apoptotic neuronal degeneration and death during the latent epileptogenic period. Such late post-traumatic pathology eventually spreads from the initial injury site to involve multiple areas of the brain [8–10]. TUNEL staining of sections from all animals collected 24 h after rLFPI also showed well-demarcated wedge-shaped regions of apoptosis that were confined to the structures underlying the craniectomy site (Fig. 1c), with no apoptotic cell death observed in the contralateral hemisphere. Semiquantified grading of FJB staining of the cortex and hippocampus 24 h after rLFPI confirmed neuronal degeneration (Fig. 1d), which was largely confined to the cortex at the lesion with minimal involvement of the underlying hippocampus. As with classic LFPI, maximal cell death was seen in the cortex near the center of the craniectomy with reduced FJB + staining near the periphery and in the ipsilesional hippocampus, whereas the contralesional cortex and hippocampus remained unaffected (Table 1).

Again, similar to classic LFPI [14,15], rLFPI consistently caused transient motor deficits, which were confirmed by testing beam walking time before and after injury. Repeated measures one-way analysis of variance indicated significant differences between preinjury and postinjury time [$F_{(5,41)} = 3.61$, $P < 0.01$]. Dunnett's multiple comparisons test showed that beam walking time increased significantly on Day 2 after rLFPI (Fig. 1e), before returning to baseline. A qualitative comparison showed that the SE as a percentage of the mean in our animals approximates that in previous reports [14,15].

Finally, we examined the long-term neuropathologic changes in the rLFPI model. Unilateral ventriculomegaly was confirmed in all animals by histological analysis in the ipsilesional hemisphere 12 weeks after injury ($n = 4$) and, in our model, was similar in size and variance to that observed in the classic LFPI model [11]. Ipsilesional lateral ventricles in our experiment were significantly larger than contralesional ventricles 12 weeks after rLFPI (2.1 ± 0.3-fold, paired *t*-test, $P = 0.036$; Fig. 1f and g), implying hippocampal volume loss, which is associated with post-traumatic cognitive decline and limbic seizures [12,13].

Table 1 Semiquantitative count of positive FJB staining in the cortex and hippocampus 24 h after rLFPI

	Cortex			Hippocampus		
Distance posterior to bregma (mm)	rLFPI IL	rLFPI CL	Sham	rLFPI IL	rLFPI CL	Sham
1	0	0	0	0	0	0
2	1	0	0	0	0	0
3	3	0	0	1	0	0
4	3	0	0	1	0	0
5	2	0	0	1	0	0
6	2	0	0	0	0	0

rLFPI results in focal injury confined to the ipsilesional hemisphere. Neuronal death is maximal in the ipsilesional cortex with minimal involvement of the ipsilesional hippocampus. Contralesional cortex and hippocampus, and sham-injured brains are unaffected.
CL, contralesional; FJB, Fluoro-Jade B; IL, ipsilesional; rLFPI, rapid lateral fluid percussion injury.

Table 2 Summary of common findings in classic LFPI and correlation with rLFPI

Feature	Classic LFPI	rLFPI
Histology: apoptotic cell death, neuronal degeneration	Yes [9,10]	Yes
Anatomy: unilateral, ipsilesional ventriculomegaly	Yes [11–13]	Yes
Motor dysfunction: increased beam walking time	Yes [14,15]	Yes
Electrophysiology: spontaneous, chronic post-traumatic seizures	Yes [16,17]	Yes [22]

LFPI, lateral fluid percussion injury; rLFPI, rapid lateral fluid percussion injury.

We also previously reported on the epileptogenicity of the rLFPI model [22], which approximates that of the classic technique [16]. In our prior report (data not shown in this manuscript), 100% of rats subjected to moderate rLFPI developed recurrent late post-traumatic seizures, as recorded by wireless EEG telemetry at 12 weeks after injury. This high prevalence of rats with seizures (defined as runs of spikes longer than 10 s on EEG) was similar to the previously reported seizure prevalence in the moderate and severe classic LFPI rat PTE models [22].

In summary, the rLFPI method provides a rapid and reproducible model of epileptogenic injury, and an effective alternative to the classic LFPI model. The LFPI PTE model is itself heterogeneous, and interanimal reproducibility may vary between laboratories depending on animal strain, the position and size of the craniectomy, and the amplitude of the fluid pulse. Although the juxtaposition of the cannula to the craniectomy rim, without cement, may introduce a mechanical variable in our procedure, we nevertheless find that rLFPI, once optimized, produces findings similar to those observed with the original technique (Table 2). Among these are acute cell injury, apoptotic cell death that continues through the subacute period, unilateral ventriculomegaly, and post-traumatic epilepsy in the chronic stage [22]. However, being a one-stage procedure, rLFPI has the advantage of enabling shorter experiment turnaround time by eliminating the need for multiple procedures and limiting the exposure to anesthetics and analgesics, which may confound interpretation of rat TBI data and carry mortality risks of its own. We are hopeful that this method will be adapted by colleagues who are working with preclinical TBI models.

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Conflicts of interest

There are no conflicts of interest.

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