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# Posttraumatic seizures and epilepsy in adult rats after controlled cortical impact

Kevin M. Kelly[a,b,c,d,*], Eric R. Miller[a], Eka Lepsveridze[e], Elena A. Kharlamov[a,b,c], Zakaria Mchedlishvili[a,b,c]

[a] Center for Neuroscience Research, Allegheny Health Network Research Institute, Pittsburgh, PA, United States
[b] Department of Neurology, Allegheny General Hospital, Pittsburgh, PA, United States
[c] Department of Neurology, Philadelphia, PA, United States
[d] Department of Neurobiology and Anatomy, Drexel University College of Medicine, Philadelphia, PA, United States
[e] Ilia State University, Faculty of Natural Sciences and Engineering, Tbilisi, Georgia

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ABSTRACT

Posttraumatic epilepsy (PTE) has been modeled with different techniques of experimental traumatic brain injury (TBI) using mice and rats at various ages. We hypothesized that the technique of controlled cortical impact (CCI) could be used to establish a model of PTE in young adult rats. A total of 156 male Sprague-Dawley rats of 2–3 months of age (128 CCI-injured and 28 controls) was used for monitoring and/or anatomical studies. Provoked class 3–5 seizures were recorded by video monitoring in 7/57 (12.3%) animals in the week immediately following CCI of the right parietal cortex; none of the 7 animals demonstrated subsequent spontaneous convulsive seizures. Monitoring with video and/or video-EEG was performed on 128 animals at various time points 8–619 days beyond one week following CCI during which 26 (20.3%) demonstrated nonconvulsive or convulsive epileptic seizures. Nonconvulsive epileptic seizures of >10 s were demonstrated in 7/40 (17.5%) animals implanted with 2 or 3 depth electrodes and usually characterized by an initial change in behavior (head raising or animal alerting) followed by motor arrest during an ictal discharge that consisted of high-amplitude spikes or spike-waves with frequencies ranging between 1 and 2 Hz. Class 3–5 epileptic seizures were recorded by video monitoring in 17/88 (19%) and by video-EEG in 2/40 (5%) CCI-injured animals. Ninety of 156 (58%) animals (79 CCI-injured, 13 controls) underwent transcardial perfusion for gross and microscopic studies. CCI caused severe brain tissue loss and cavitation of the ipsilateral cerebral hemisphere associated with cell loss in the hippocampal CA1 and CA3 regions, hilus, and dentate granule cells, and thalamus. All Timm-stained CCI-injured brains demonstrated ipsilateral hippocampal mossy fiber sprouting in the inner molecular layer. These results indicate that the CCI model of TBI in adult rats can be used to study the structure–function relationships that underlie epileptogenesis and PTE.



## 1. Introduction

According to the Centers for Disease Control and Prevention, 1.7 million Americans sustain traumatic brain injury (TBI) each year. Of these individuals, 52,000 die, 275,000 are hospitalized, and 1365 million are treated and released from an emergency department (Faul et al., 2010). An estimated 1.1% of the U.S. civilian population lives with a long-term disability from TBI (Zaloshnja et al., 2008). One of the sequelae of TBI is the development of posttraumatic epilepsy (PTE). In a population-based study, the cumulative incidence of PTE in the first three years after hospitalization per 100 persons was 4.4 for mild TBI, 7.6 for moderate TBI, and 13.6 for severe TBI (Ferguson et al., 2010). The risk of PTE is especially high in the military, where the incidence can be up to 53% after penetrating head trauma (Raymont et al., 2010). Although the link between head trauma and seizures has been recognized for millennia, successful animal modeling of TBI and the subsequent development of PTE has occurred only within the recent past

*Abbreviations:* TBI, traumatic brain injury; PTE, posttraumatic epilepsy; CCI, controlled cortical impact; FPI, fluid percussion injury; POD, postoperative day; SWD, spike-wave discharges.

* Corresponding author at: Allegheny General Hospital, 940 South Tower, 320 E. North Avenue, Pittsburgh, PA 15212-4772, United States. Tel.: +1 412 359 3467; fax: +1 412 359 6127.
*E-mail addresses:* kelly@wpahs.org (K.M. Kelly), emiller@wpahs.org (E.R. Miller), eka_lepsveridze@iliauni.edu.ge (E. Lepsveridze), zmchedlishvili@sentientmedical.com (Z. Mchedlishvili).

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(D'Ambrosio et al., 2004a,b; Pitkänen and McIntosh, 2006; Statler et al., 2009; Hunt et al., 2009; Pitkänen et al., 2009, 2011; Curla et al., 2011; Bolkvadze and Pitkänen, 2012). Building on the substantial database of clinical, experimental, and pathological findings associated with TBI, biological mechanisms underlying PTE are actively being explored (D'Ambrosio et al., 1999; Norris and Scheff, 2009; Prince et al., 2009; Willmore and Ueda, 2009; Hunt et al., 2010, 2011; Kharatishvili and Pitkänen, 2010a; Mtchedlishvili et al., 2010; Kharlamov et al., 2011; Belousov et al., 2012; D'Ambrosio et al., 2013a,b; Hunt et al., 2013).

Experimental models of TBI simulate human TBI with varying degrees of accuracy (Morales et al., 2005). Two of the most commonly used models of TBI are fluid percussion injury (FPI) and controlled cortical impact (CCI). The advantage of the FPI model is its relative simplicity and its ability to produce significant injury in the brain, including axonal injury and intraparenchymal hemorrhages (Povlishock et al., 1983). The FPI model has been used successfully to demonstrate the development of PTE (D'Ambrosio et al., 2004a,b; Kharatishvili et al., 2006; Kharatishvili and Pitkänen, 2010b; Bolkvadze and Pitkänen, 2012). Alternatively, CCI produces a relatively precise and reproducible injury and can simulate various degrees of TBI severity by titration of the velocity, force, and depth of impact (Dixon et al., 1991). The CCI model has been well characterized histopathologically (Lighthall, 1988; Hall et al., 2005; Saatman et al., 2006) and has been used successfully in mice (Hunt et al., 2009, 2010) and in postnatal day 7 rats to demonstrate the development of PTE (Statler et al., 2009). We hypothesized that the technique of CCI could be used to develop a model of PTE in young adult rats. The main objectives of this study were to characterize: (1) the frequency and severity of both acute symptomatic seizures (i.e., provoked, occurring within the first week following acute brain injury) and epileptic seizures (i.e., unprovoked, occurring after the first week following acute brain injury); (2) the electrographic signatures of epileptogenesis and the epileptic state; and (3) the associated neuropathological changes that occurred following extended periods of animal survival after CCI.

## 2. Materials and methods

### 2.1. Controlled cortical impact (CCI)

All procedures involving animals were approved by the Institutional Animal Care and Use Committee of the Allegheny Health Network Research Institute and were conducted according to NIH guidelines and regulations. Animals were housed individually in the ASRI vivarium, maintained in a 12 h light/12 h dark cycle environment with controlled temperature (23 ± 2 °C), and food and water were given ad libitum. The CCI procedure was performed according to Dixon et al. (1991) with some modifications (Mtchedlishvili et al., 2010) using aseptic technique. Briefly, male Sprague-Dawley rats (2–3-mo old) were anesthetized with an initial dose of 4% isoflurane mixed with oxygen and positioned in a stereotaxic frame (David Kopf Instruments, Tujunga, CA); surgical depth of anesthesia was maintained with 2–3% of isoflurane. Body temperature was monitored throughout the procedure using a rectal probe and maintained at 37 ± 2 °C with a heating pad (Harvard Apparatus). A craniectomy was performed over the right parietal cortex within the boundaries of the bregma and lambda while leaving the dura intact. CCI was performed immediately after removal of the bone flap and exposure of the dura. A 1.975-cm-diameter pneumatic impactor, attached to a double-acting, stroke-constrained, pneumatic cylinder with a 5.0 cm stroke (Pittsburgh Precision Instruments, Pittsburgh, PA) was used to deliver CCI. The impactor tip was positioned 4–5 mm laterally to the midline. Following initial titration of the impact procedure, for the majority of animals ($n = 116$) the impactor velocity was adjusted to 4 m/s, the impact duration was 100 ms, and the depth of tissue depression was 2.8 mm (Mtchedlishvili et al., 2010; Kharlamov et al., 2011). Following CCI and the cessation of cortical bleeding, the scalp was sutured, carprofen 5 mg/kg was administered subcutaneously, and the animal was returned to the vivarium for recovery or to satellite vivaria of the Neurophysiology Laboratory for video monitoring for up to 7 days post-lesioning. Carprofen use was continued each day for 48–72 h as judged necessary for recovery. Sham-operated animals underwent identical anesthetic and surgical procedures without CCI.

### 2.2. Electrode implantation

Cortical and hippocampal recording depth electrodes and anchoring screws were placed about one week after CCI or sham operation, or at various time points during extended video-only monitoring, as previously described for the photothrombosis method (Kelly et al., 2001), with modifications. Animals were anesthetized with intraperitoneal injections of a 9:1 mixture of ketamine and xylazine, and after the loss of the tail pinch reflex, positioned in a stereotaxic frame. A sterile artificial tear ointment was applied over the eyes and a midline incision was made along the scalp, which was reflected bilaterally. Burr holes were drilled for 3 depth electrodes (0.25 mm; Plastics One Inc., Roanoke, VA), 1 reference electrode, and 4–5 anchoring screws using stereotactic coordinates. Cortical depth electrodes were placed rostral to the lesion and homotopically (AP 3.2 mm, lateral 1.5 mm, vertical −3.5 mm). A hippocampal depth electrode was placed in the ventral hippocampus contralateral to the injury (AP −5.3 mm, lateral 4.9 mm, vertical −6 mm to dura). A hippocampal electrode was not placed on the side of the lesion due to the craniectomy defect. A single skull screw electrode overlying the cerebellum was used as a reference electrode. Anchoring screws were placed posterolaterally; one anchoring screw was used as a ground electrode. All electrodes were crimped into a six-conductor modular plug and secured to the skull surface with dental acrylic (Lang Dental Mfg., Wheeling, IL). The skin around the headset was sutured and bipolar EEG recordings were initiated following full recovery of the animal.

### 2.3. Video and video-EEG monitoring

Animals were housed individually in 12 monitoring chambers in satellite vivaria of the Neurophysiology Laboratory and maintained on the 12 h light/12 h dark cycle. Animal behavior was variably monitored around-the-clock by closed-circuit television cameras that were connected to video splitter units (Advanced Technology Video, Inc., Redmond, Washington). Digital video files (Diva, Stellate Systems) were recorded directly to high capacity hard disk drives using removable hard drive bays. Daily recordings were reviewed visually offline to detect any behavioral seizure activity according to a modified classification scale (Racine, 1972), including forelimb clonus (class 3); running and rearing (class 4); and jumping and falling (class 5). Seizures that occurred during the first week following CCI were considered provoked seizures; these animals underwent video-only monitoring. Seizures that occurred after the first week following CCI were considered epileptic (spontaneous) whether they were isolated or recurrent events, consistent with the proposed conceptual definition of epilepsy by the International League Against Epilepsy as "an enduring alteration in the brain and at least one seizure" (Fisher et al., 2005).

Specific criteria for video and video-EEG was not adopted for the first 30 animals, for which monitoring was sporadic, i.e., varying numbers of days monitored per month for 3 months, and was skewed to specific CCI-injured animals that displayed potentially abnormal behavior (12/30; 40%). We commenced acute, post-CCI