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A Potential Model of Pediatric Post-traumatic Epilepsy

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Abstract

Preclinical models of pediatric post-traumatic epilepsy (PTE) are lacking. We hypothesized that traumatic brain injury (TBI), induced by controlled cortical impact, in immature rats would cause electroencephalographic (EEG) epileptiform activity and behavioral seizures. TBI or sham craniotomy was performed on postnatal day 17. Using video-EEG monitoring 4-11 months post-TBI, most TBI rats (87.5%) showed EEG spiking and one had spontaneous, recurrent seizures. Controls showed neither EEG spikes nor electrographic/behavioral seizures. Late seizures were rare after TBI, but EEG spiking was common and may represent a surrogate for PTE.

Introduction

Posttraumatic epilepsy (PTE), defined as recurrent, unprovoked seizures after traumatic brain injury (TBI), is a devastating sequela for 10-20% of children suffering severe TBI (Appleton and Demellweek, 2002; Barlow et al., 2000). PTE pathogenesis is poorly understood and preclinical models are limited (Pitkanen and McIntosh, 2006). We hypothesized that TBI in immature rats would induce EEG epileptiform activity and spontaneous seizures later in life.

Methods

Litters of Sprague-Dawley rats (Charles River Laboratories, Raleigh, NC) were housed with a lactating female until weaning on post-natal day (PND) 21, then segregated and housed by gender in a temperature- and light-controlled (12 h on/12 h off) environment. Mature rats had free access to food and water. Our local Institutional Animal Control and Use Committee approved all animal care and experimental interventions.

Rats underwent controlled cortical impact (CCI) centered over the left parietal cortex (4-mm rostral to lambda and 4-mm left of midline, 5-mm rounded tip, 4 m/sec velocity, 2-mm deformation, and 100-msec duration; $n=5$ male, 5 female) or sham craniotomy ($n=3$ male, 3 female) using isoflurane anesthesia on PND 17. The bone flap was not replaced after either

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TBI or craniotomy. A single-channel epidural EEG electrode was implanted at the caudal edge of the TBI lesion (1-mm rostral and 1-mm left of bregma) using isoflurane anesthesia on PND 90-180. All surgical procedures were performed using aseptic technique.

Video-EEG monitoring was initiated 4-8 months post-TBI and continued for 3 months using a single-channel tethered EEG recording system (Plastics One, Roanoke, VA). Continuous (24/7) recording was conducted for serial 2-8 week epochs. EEG signals were acquired using AcqKnowledge acquisition software (BioPac Systems Inc.; Santa Barbara, California). Video images were recorded using a standard video digital surveillance system with infrared cameras to enable night recording (Automated Video Systems, Woods Cross, UT). Monitoring was discontinued if the EEG electrode ceased functioning or if electrical noise prohibited a reliable EEG signal.

Single observers blinded to experimental group analyzed EEG and video data (PS and KS respectively). *A priori* definitions were: EEG spiking = distinct spikes ≥ 3 times baseline with duration 20-70 msec; EEG seizure = repetitive spiking that persists >15 sec; posttraumatic seizure = EEG seizure confirmed by motor seizure. Behavioral seizures were rated by modified Racine scale (Racine, 1972).

After monitoring, rats were anesthetized and perfused. Brains were removed, post-fixed, cryoprotected, and then blocked and embedded in paraffin. The entire cerebral hemispheres were sectioned into 10-um coronal slices and stained with hematoxylin and eosin. A section selected at random from within the first 100-um was digitized and the perimeters of the entire brain, the lesion, and the right and left hippocampus were traced using StereoInvestigator Version 8 software (MicroBrightField, Williston, Vermont). Subsequently, serial sections were selected every 100-um and similarly digitized throughout the entire hemisphere. Hippocampal and lesion volumes were determined using the standard volumetric analysis option in the software package.

Physiological and histological parameters were compared by Mann Whitney test. Rat weights were compared by multiple analysis of variance. Statistical analyses were performed using SPSS 11 for Mac (Chicago, IL). A p-value of < 0.05 was considered significant.

Results

TBI and control rats had similar anesthetic durations and weight gain during the experimental period (data not shown). Mortality was 10% for TBI and 0% for control rats. Noise artifact limited EEG recordings in one TBI and 2 control rats; 8 TBI and 4 control rats were analyzed.

Mean ($\pm$ SD; range) durations of continuous (24/7) video-EEG monitoring for TBI and control rats were 49 (± 35 ; 19-90) and 15 (± 18 ; 9-41) days. EEG spiking was seen in all but one TBI rat (87.5%) (Fig. 1A) and occurred on 4-12% of monitored days. EEG activity questionable for seizure was identified in all TBI rats that had spiking. To be confirmed as a seizure, either simultaneous convulsive motor activity or clear characteristic class 1 or class 2 behaviors had to be present (Racine, 1972). Behavioral seizure confirmation was observed in one TBI rat. That rat had a total of 4 seizures (Fig. 1B); each lasted 45-60 sec and met criteria for Racine (1972) class 4-5 with generalized clonic motor activity. The first was observed during the initial week of monitoring (post-injury day 260). Three were observed over a 2-day period. EEG spiking was most frequent in the TBI rat with seizures. Spike morphology was similar for TBI rats with and without seizures. No control rat had any EEG spiking or seizures.

TBI induced a lesion to left parietal cortex [mean ($\pm$ SD) volume 23.9 ($\pm$ 7.6) mm³]. The rat with posttraumatic seizures had no evidence of cerebral infection on gross or histological evaluation and a similar lesion size to other TBI rats. Mean hippocampal volumes did not differ for TBI and control rats [left: 15.7 ($\pm$ 4.2) mm³ vs. 19.3 ($\pm$ 6.6) mm³; right: 22.4 (1.4 $\pm$) mm³ vs. 22.1 ($\pm$ 6.1) mm³; p = 0.4)]. Control and TBI rats had a similar degree of fibrous healing over the craniotomy site. Surprisingly, control rats also showed some lesion of left parietal cortex [mean volume 10.8 ($\pm$ 10.2) mm³, smaller than that seen in TBI rats (p < 0.05)], likely related to maturation with a skull defect following sham craniotomy.

Discussion

We observed EEG spiking in 87.5% of TBI rats and spontaneous, recurrent seizures in one TBI rat. These preliminary findings suggest CCI during immaturity as a possible model for pediatric PTE. In corroboration, behavioral seizures (modified Racine scale grade 2-3) have recently been reported in 20-36% of adult mice post-CCI (Hunt et al., 2009).

Further, our findings emphasize some important challenges in PTE research. First, only one of 8 TBI rats showed convulsive seizures, suggesting a low prevalence of PTE and/or a low seizure frequency. Second, the first observed seizure occurred 260 days post-TBI; however, continuous monitoring is required to determine the latent period. Third, observed seizures appeared to cluster. Finally, it is possible that some TBI rats had non-convulsive seizure activity, although rigorous assessment of non-convulsive seizures was beyond the scope of our study. Our data suggest the need for large sample sizes and more sustained (if not continuous) long-term monitoring. The use of >1 EEG electrode (preferably both surface and hippocampal depth electrodes) would also be necessary to localize seizure onsets. Clustering, long latent periods (2-12 months), non-convulsive seizures and hippocampal seizures have been reported in mature rats with PTE after fluid percussion TBI (D'Ambrosio, et al., 2005; Kharatishvili et al., 2006).

The low seizure incidence, clustering, and long latent periods suggested by our study also indicate need for a surrogate marker of PTE. EEG spiking is one possibility. We observed EEG spiking in all except one TBI rat, but in no control rats. Further, EEG spiking was most frequent in the rat that had PTE. EEG spiking is used clinically to identify an epileptic focus, to indicate seizure risk, and to guide antiepileptic therapy. Similar to using EEG spiking as an *in vivo* surrogate, spontaneous bursting in hippocampal slices may correlate with behavioral seizures after CCI (Hunt et al., 2009). Further investigation into the feasibility of EEG spiking as an *in vivo* surrogate marker of PTE is needed.

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Figure 1. Posttraumatic EEG spikes and seizures

Representative records of EEG spikes and seizures. The numbers above each time-compressed signal correspond with time-expanded segments beneath. **(A)** EEG spiking in a TBI rat without detected convulsive seizures. Spike morphology was similar for TBI rats with and without seizures. **(B)** EEG seizure from the rat with recurrent, spontaneous posttraumatic seizures. Generalized clonic motor activity (Racine class 4-5) accompanied this EEG seizure.