Increased Expression of Epileptiform Spike/Wave Discharges One Year after Mild, Moderate, or Severe Fluid Percussion Brain Injury in Rats

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Abstract

In this study, we describe increased expression of cortical epileptiform spike/wave discharges (SWD) in rats one year after mild, moderate, or severe fluid percussion traumatic brain injury (fpTBI). Groups of rats consisted of animals that had received mild, moderate, or severe fpTBI, or sham operation one year earlier than electrocorticography (ECoG) recordings. In addition, we included a group of age-matched naïve animals. ECoG was recorded from awake animals using epidural electrodes implanted on the injured hemisphere (right), sham-operated hemisphere (right), or right hemisphere in naïve animals. The SWDs were detected automatically using Fast Fourier Transformation and a novel algorithm for comparing changes in spectral power to control (nonepileptical) ECoG. The fpTBI resulted in increased expression of SWDs one year after injury compared with sham-operated or naïve animals. The number of SWD-containing ECoG epochs recorded in a 1 h recording session were: naïve 12.9 ± 10.3 , $n=8$, sham 23.6 ± 8.2 , $n=10$, mild TBI 78.9 ± 23.9 , $n=10$, moderate TBI 61.3 ± 32.5 , $n=12$, severe TBI 72.5 ± 28.3 , $n=11$ (mean $\pm$ standard error of the mean). Increased expression of SWDs was not related to injury severity. SWDs were observed to a lesser extent even in sham-operated and naïve animals. The data indicate that fpTBI exacerbates expression of SWDs in the rat and that this increase may be observed at least one year after injury. As others have discussed, the spontaneous occurrence of these epileptiform events in rodents limits the use of this model for investigations of acquired epilepsy, at least of the nonconvulsive type, after TBI.

Keywords: electrocorticogram; electroencephalogram; epilepsy; fluid percussion; nonconvulsive seizures; spike wave discharges; traumatic brain injury

Introduction

ACQUIRED EPILEPSY is a well-recognized secondary neurological complication resulting from a variety of brain injuries including traumatic brain injury (TBI), stroke, subarachnoid hemorrhage, and tumor formation.¹ The association between TBI and seizures has been appreciated for decades² and confirmed by multiple clinical studies up to the present time.^{3,4} A consensus from these studies is that not only does TBI increase the risk of epilepsy developing but also that this risk increases with injury severity.⁴ In addition to the increased risk of convulsive seizures after TBI, there is also increased risk of electrographical epileptic activity developing without behavioral convulsions. These have been termed nonconvulsive seizures, which also increase after TBI.⁵

Because of the clinical importance of post-traumatic epilepsy, there is a considerable body of research in animal models examining

the cellular and molecular basis of this form of acquired epilepsy and the similarities and dissimilarities between animal models and human post-traumatic epilepsy. Much of this work has been reviewed elsewhere.⁶ As in humans, the incidence of post-traumatic seizures in rodents correlates with injury severity and whether injury involves direct penetrating damage to the brain.⁶ Thus, in rodents, severe fluid percussion TBI (fpTBI) increases the incidence of convulsive seizures more than either mild or moderate fpTBI, and cortical impact injury or penetrating ballistic injury increases the incidence of convulsive seizures more than either fp injury or closed skull concussive injury.^{6,7} In addition to increasing the likelihood of spontaneous convulsive seizures, TBI has been shown also to lower the threshold for elicitation of convulsive seizures by chemical^{8–10} or electrical stimulation,^{10,11} suggesting TBI-induced circuit hyperexcitability.

Experimental examination of nonconvulsive seizures in rodent models of TBI has been limited. D'Ambrosio and associates^{12,13}

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reported an increase in electrographical epileptic activity with behavioral freezing. Campbell and colleagues¹⁴ also reported increased electrographical epileptic activity without convulsions after severe fp injury. In contrast, Rodgers and coworkers¹⁵ found no significant increase in epileptic activity after moderate and severe fpTBI in rats. To date, most studies of nonconvulsive seizure activity after TBI have not examined chronic time points that would indicate long-term changes leading to brain hyperexcitability and consequently epilepsy.

The goal of this study was to examine the effects of mild, moderate, and severe TBI on electrographical activity and behavior of rats one year after brain injury. We report an increase in the expression of epileptiform spike/wave activity accompanied by freezing behavior after fp brain injury that was not dependent on injury severity. Some of these findings have been reported in preliminary form.¹⁶

Methods

Animals and fp brain injury

All procedures involving animals were conducted using the guidelines and approval of the University of Miami Animal Care and Use Committee.

The experimental animal groups for this study consisted of Sprague-Dawley rats that had undergone mild, moderate, or severe fpTBI, or sham injury, at three months of age. At the time of injury, animals were assigned to one of these experimental groups, and surgical procedures were conducted randomly. Animals were given a code number based on the day of TBI operation. Age and strain-matched injury-naïve rats were also used, but these animals were acquired two weeks before any procedure for electrophysiology.

Details of fp injury, as conducted in our laboratory, have been published previously.¹⁷ Briefly, injury consisted of a two-stage procedure under isoflurane anesthesia. During the first stage, a 4.8 mm craniotomy was performed over the parietal cortex for attachment of a plastic injury tube. Twenty-four hours later, the animals were re-anesthetized, and the injury tube was attached to the fp injury device. The animals were intubated and mechanically ventilated while monitoring of arterial blood pressure and periodical sampling of blood for blood gas measurements was conducted. Animals were grouped as mild, moderate, or severe fpTBI based on the characteristics of the fp wave: mild 1.2–1.5 ATM, moderate 1.8–2.2 ATM, severe 2.4–3.0 ATM. Sham animals received all procedures without delivery of the fp pulse. Age-matched naïve animals did not undergo any injury-related procedure. After the procedure, animals were returned to the vivarium and housed there under controlled light/dark and temperature/humidity conditions for one year. One-year-old surgical procedure-naïve rats were acquired and housed for two weeks before electrocorticography (ECoG) recordings.

Electrophysiology

One year after fpTBI or sham operation, animals were prepared for monitoring of awake ECoG activity. The ECoG recordings were conducted in injured and sham animals within three days of the anniversary of their injury and thus remained randomized for ECoG recordings. Age-matched naïve animals were treated similarly except that the animals were implanted for recordings at random intervals with the other groups of animals. Laboratory personnel conducting the recordings were only given animal code numbers and thus were blinded to the animal group assignment.

Animals were anesthetized with isoflurane (4% induction, 2% maintenance) and placed in a stereotaxic head holder (Kopf). A 2-cm midline incision was made and blunt dissection was used to carefully expose the cranial surface. Two small (2-mm) burr holes were drilled to expose the dura anterior (bregma - 1.0 mm, 2 mm

lateral to midline) and posterior (lambda +2.0 mm, 2.0 mm lateral to midline) to the previous TBI craniotomy site. Two silver-silver chloride coiled wire electrodes (2-mm coil diameter) were gently placed in the two burr holes and attached to the base of a custom-made magnetic head stage containing gold pin connectors (Apple MagSafe laptop connector). A third indifferent electrode, which served as ground, was implanted into muscle lateral to the contralateral hemisphere. The head stage and implanted electrodes were attached permanently to the skull with dental acrylic, and the opening through the skin was closed around the head stage with surgical staples. Animals were allowed to recover from surgery for 24 h before recordings.

Recordings of ECoG activity were made in awake animals using a homemade head stage that provided 10X amplification of the differential signal between the two implanted electrodes. The head stage amplifier provided high pass filtering above 0.1 Hz. The head stage contained the mating magnetic connector (Apple MagSafe laptop connector) that allowed rapid and stable connection of the amplifier head stage to the electrode head stage implanted in the animals. The amplifier head stage received power from, and transmitted ECoG signals to, a CWE Model 820 second stage conditioning amplifier. This amplifier provided additional 100X amplification and high frequency cutoff filtering at 500 Hz. The amplified and conditioned signals were digitized and recorded with AD Instruments Powerlab and Labchart software, respectively. Simultaneous video recordings were made to correlate ECoG signals with animal behavior. In some animals, behavior was quantified using Ethovision software to monitor animal movement. Recordings were made from each animal for 1 h.

ECoG seizure detection was performed with customized software¹⁸ developed to modify the EEGLab module using the MatLab platform. In brief, the goal was to quantify ECoG events for each 1 h recording period using short time period Fourier transformation (STFT). The procedure is summarized as follows: average spectral power was measured from a 4 min segment of ECoG obtained from aged-matched naïve animals during awake exploratory behavior (typified by high frequency, low amplitude ECoG activity).

Power in the major frequency bands (delta 0.5–3.5 Hz, theta 3.5–7.5 Hz, alpha 7.5–12.5 Hz, beta 12.5–30 Hz, and gamma rhythms 30–60 Hz) was calculated using STFT for each 4 sec period of ECoG activity for the entire 1 h recording period. All power measurements were normalized using a median decaying memory algorithm¹⁹ to control for possible differences in absolute ECoG amplitudes because of differences in signal strength.

ECoG epochs containing spike/wave discharges (SWDs) were detected by first comparing power in each frequency band with the power observed in controls as described above. A significant change in power was detected when power in any frequency band exceeded that of the control ECoG power by three standard deviations. Then, each ECoG epoch was examined for a particular pattern of significant frequency changes that were unique to SWDs. Preliminary comparisons of power changes in each frequency band with known SWD containing ECoG records indicated that SWDs would be reliably detected when power significantly increased in a combination of the theta, alpha, beta, and gamma frequency bands. Any ECoG epoch having increased power in this frequency band combination was scored as an epoch containing the SWD pattern.

Once the pattern was determined, all ECoG records for all animals were processed, without experimenter intervention, to detect the total number of SWD-containing epochs in a 1 h period. This analysis did not attempt to score the number of SWDs or their duration, and by not doing so, did not place any particular weight on short or long duration SWDs. To examine possible differences in durations of SWDs, the number of consecutive ECoG epochs that contained SWDs was determined. Thus, a single SWD containing epoch not followed by another epoch was considered to have a duration of 2 sec or less. Two consecutive SWD containing epochs was considered to have a duration of 2–4 sec and so on.

Recording sessions consisted of placing each animal in a novel environmental chamber for 1 h. We thought this was preferable to a longer recording, because each animal was subjected to the identical start and end point of the session as defined by the experimenter rather than allowing the animals to undergo various changes in behavioral state without experimental control. All recordings were conducted during the dark cycle of the animal's light/dark circadian rhythm in a dimly lit room.

Histology

Estimates of TBI induced damage to selected brain structures was conducted by volumetric analysis as described previously.²⁰ Coronal sections at multiple levels (0.8, 1.8, 3.3, 4.3, 5.8, 6.8, and 7.3 mm posterior to bregma) were used for volumetric measurement. Volume measurements for each structure were computed using area components from multiple semiserial sections using numerical integration of successive areas. Areas were determined by tracing the boundaries of each structure at a power of $1\times$ from a minimum of three coronal sections at different bregma levels depending on the size of the structure. Volumes were expressed as the ratio of structure volumes from right and left hemisphere

Statistics

Statistical comparisons among groups were made using one-way analysis of variance (ANOVA) and Student *t* statistic. Three animals (one naïve and two shams) were removed from analysis because of acute cortical injury resulting from ECoG electrodes or skullcap mounting screws. Distributions of SWD durations were compared using the Kolmogorov-Smirnov nonparametric test.

Results

In terms of behavior, overt spontaneous clinical seizures consisting of myoclonus or tonic-clonic seizures were not observed in any animals regardless of injury severity during the 1 h recording period. Animals showed normal exploratory behavior and rarely periods of sleep. The most distinctive behavior, however, consisted of a freezing-like posture that would last from a few seconds to as long as 20 sec. Animals showing freezing abruptly stopped moving or exploring and remained stationary in an upright position. This behavior was quantified in some animals by measuring head acceleration from videos timed locked to ECoG records. Figure 1 shows an example of behavioral freezing and the corresponding ECoG analysis. In this example, a SWD lasting about 15 sec was associated with a cessation of locomotor activity.

Three ECoG activity patterns were observed during a 60 min recording period in awake one-year-old animals. These are shown in Figure 2 with corresponding power spectrograms (Fig. 2 top) and associated power spectra (Fig. 2 bottom). The first pattern consisted of low amplitude ECoG activity with most power distributed in the frequency bands below 10 Hz. Animals showing this ECoG pattern always demonstrated active exploratory behavior. The second pattern consisted of high amplitude waves containing both slow wave and sharp wave patterns with power distributed in low and mid frequency bands (generally 20 Hz and lower). A key feature of these events was their relative sudden onset and offset. We refer to these events as hypersynchronous discharges (HSDs). During these episodes animals typically stopped exploratory behavior and became immobile.

The third and most epileptic-like pattern consisted of high amplitude SWDs. Power analysis showed increased power in low, mid, and high frequency bands that most often showed a banding feature with power concentrated in bands near 8 Hz, 15 Hz, 23 Hz,

FIG. 1. Examples of electrocorticography (ECoG) traces recorded during awake exploring behavior and during a cortical spike/wave discharge (SWD). **A** and **B** are expanded time scale tracings of time points i and ii in the contracted time scale ECoG trace shown in **C**. **D** is a measurement of animal movement recorded from a video recording time-locked to trace **C**. The animal movement record shows typical freezing behavior associated with the SWD.

and 32 Hz, but with increased power up to approximately 80 Hz. During SWDs, animals also stopped exploratory behavior and became immobile. These events were automatically detected and quantified for comparison among naïve, sham, and TBI groups. We did not attempt to quantify HSDs because these events were not considered to be "epileptiform."

Expression of epileptiform spike/wave activity after *fpTBI*

ECoG analysis was conducted from 60 min recordings from animals that had mild, moderate, or severe TBI, or sham operation one year earlier or age-matched naïve animals. Figure 3 shows an example of the analysis of SWDs using this method.

The top trace is the raw ECoG record. The next six traces correspond to the normalized changes in power in the delta, theta, alpha, beta1, beta2, and gamma bands for the entire 60 min recording period. Missing power values in the first few minutes of the recording are the result of the median decaying memory algorithm. The dots above each power band indicate an increase in power exceeding normal exploratory behavior by three standard deviations. Each dot represents a 4 sec epoch from which power was calculated using fast-Fourier transformation. The SWDs were characterized by increased power in all frequency bands but most prominently in theta, alpha, beta1, beta2 and gamma bands. Occasionally, SWDs were observed without increased power in the lowest (delta) frequency band, so increased power in this band was not required for detection. Events included for quantitation of SWDs among experimental groups were required to have significantly increased power inclusively in the theta through gamma bands.

FIG. 2. Top panel: another example taken from an animal one year after mild traumatic brain injury showing expanded electrocorticography (ECoG) recordings sampled during awake exploration (A), during a freezing-associated spike/wave discharge (SWD) (B), and during another common ECoG pattern referred to as a hypersynchronous discharge (HSD) that was distinct from either the exploratory ECoG pattern or the SWD pattern. Trace D shows a more compacted 10 min segment of ECoG that contained exploratory behavior (i), six repeated SWDs (ii), and a period of HSDs. The panel in E shows a spectrogram of ECoG power at various frequencies that is time-locked to the ECoG trace shown in D. Lower panel: examples of power spectra acquired during exploration (i), SWD (ii), and HSD (iii).

The incidence of SWD-containing ECoG epochs was highly variable among animals in each of experimental groups ranging from zero to as many as 320 in a 1 h recording period. For reference, 60 SWD-containing epochs reflects 240 sec of ECoG or 4 min of the 60 min recording period. The mean number of SWDs/60 min for the experimental groups was naïve 12.9 ± 10.3 , $n = 8$; sham 23.6 ± 8.2 , $n = 10$; mild TBI 78.9 ± 23.9 , $n = 10$; moderate TBI 61.3 ± 32.5 , $n = 12$; severe TBI 72.5 ± 28.3 , $n = 11$ (mean $\pm$ standard error of the mean). There was no significant difference between naïve and sham animals, and no significant difference among the

injury severity groups. Therefore, naïve and sham groups were pooled as were the mild, moderate, and severe TBI groups for statistical comparisons. This comparison is shown in Figure 4. Injured animals showed a significant increase in the number of SWDs compared with noninjured animals (Student t test, $p = 0.012$).

The durations of SWDs were examined in all animals to determine whether TBI either prolonged or shortened these discharges. Figure 5 shows the fraction of SWDs of different durations in the combined naïve/sham groups and the combined TBI groups. In

FIG. 3. Example of an electrocorticography (ECoG) record from an animal one year after moderate fluid percussion traumatic brain injury (top trace). The lower six traces show changes in normalized power for delta, theta, alpha, beta1, beta2 and gamma frequency bands. The red dots indicate ECoG epochs during which power in each band exceeded the power recorded during exploratory behavior by three standard deviations. These events were used to quantitate the occurrence of spike/wave discharge in each ECoG record (see Methods).

both groups, the majority of SWDs (greater than 50%) had durations less than 2 sec. In both groups, fewer SWDs were observed as the duration increased. Statistical comparison of the distributions of SWD durations between the two groups indicated that they were not different ($p=0.43$)

FIG. 4. Quantitation of spike/wave discharge (SWD)-containing electrocorticography (ECoG) epochs from rats one year after mild, moderate, or severe fluid percussion traumatic brain injury or sham injury, and age-matched naïve rats showing increased numbers of SWDs in injured animals compared with uninjured controls. Because there was no statistical difference among the injury severity groups, or between naïve and sham-operated animals, the injury groups and the sham/naïve groups, respectively, were pooled. The insert shows pooled data. * Student *t* test indicated a significant difference between the uninjured and injured groups ($p=0.012$)

Histology

There was no significant difference in the incidence of SWDs in the three injury severity groups. In fact, more SWDs were seen in animals with mild TBI than in either the moderate or severe TBI animals. It was important, therefore, to verify injury severity by histological analysis at one year. Animals undergoing mild, moderate, or severe injury showed extensive loss of cortical volume (ANOVA, $p<0.05$) and volume of the external capsule (ANOVA $p<0.001$). Hippocampal volume appeared to decrease mostly after severe TBI but was significantly different among the groups (ANOVA, $p<0.05$). Figure 6 shows quantitative analysis of cortical volume in the three injury groups. These data suggest that cortical injury might be sufficient to increase the incidence of SWD expression and that increasing injury severity would not worsen this condition.

Discussion

This study reports that the occurrence of SWDs was increased ONE year after fp brain injury in rats and that this increase was independent of injury severity. A few previous studies have investigated the effect of fp injury on epileptiform SWDs in rats^{12–15,21} and have considered these events to be a form of nonconvulsive epilepsy. Early work by D'Ambrosio and associates^{12,13} and Curia and colleagues²¹ found an increase in electrographical epileptic discharges associated with behavioral freezing two weeks after severe TBI. The incidence of these events decreased with time post-injury up to approximately 19 weeks.^{12,21} Campbell and coworkers¹⁴ also reported an increase in the frequency of non-convulsive seizures after severe TBI. Unlike the earlier studies, however, they reported an increase in nonconvulsive seizure 33

FIG. 5. Fraction of spike/wave discharge (SWDs) of increasing duration recorded in naïve/sham and traumatic brain injury (mild + moderate + severe) animals. The majority of SWDs in both groups were less than 2 sec in duration, with fewer SWDs observed as the duration increased. There was no significant difference in the distributions between the two groups ($p=0.43$, Kolmogorov-Smirnov test for equality of two distributions)

weeks after severe TBI. We also report an increase in epileptiform SWDs associated with behavioral freezing 52 weeks after TBI. We further observed this increase in rats receiving mild, moderate, or severe TBI.

Although a number of research groups, including ours, have reported increases in SWDs after fp brain injury in rats, a careful study by Rodgers and colleagues¹⁵ failed to detect any differences between control animals and animals receiving either moderate or severe TBI. They examined SWDs in rats of a variety of ages. An important conclusion of this work was that rats without brain injury spontaneously display SWDs and that the occurrence of these events increases with age. This is particularly important for our study in which the expression of SWDs was examined one year after injury.

The age dependence of SWDs has been reported as well by others albeit in other rat strains used as models of absence epilepsy.^{22,23} The spontaneous occurrence of SWDs and their age dependency complicates interpretation of studies of nonconvulsive seizures after TBI or other forms of brain injury. For example, it is not possible to determine from our data, or the data from other investigators showing TBI-induced increases in SWDs, whether TBI resulted in conversion of normal brain to epileptic brain or resulted in alterations of brain excitability that allowed increased expression of SWDs in brain already expressing these events. To do so will require comparison of the effects of TBI in animals that show spontaneous SWDs with those that do not. The age dependence of SWD expression in rats also makes it difficult to interpret acquired epileptogenesis after TBI because the time course of this acquired epilepsy and the normal age dependence are so similar.

While the focus of our investigation was on effects of fp brain injury on nonconvulsive seizures, it is important to consider that other forms of brain injury have been associated with nonconvulsive seizures and electrographical SWDs. One such injury is focal cortical injury resulting from focal cortical thrombosis.^{24,25} The SWDs have also been associated with injury after cortical impact,²⁶ ballistic penetrating injury,²⁷ and stroke by middle cerebral artery occlusion.^{27,28} As with fp injury, these cortical injury studies noted spontaneous SWDs and freezing behavior in uninjured controls. Whether spontaneous SWDs in rodents are good

model of absence seizures has been discussed previously.²⁹ Also, a discussion of spontaneous SWDs in rodent models of brain injury with cautions about the necessity for adequate controls and possible methods for reducing confounding has been provided recently.²³

While studies of SWDs in rodents have not provided conclusive evidence that TBI causes *de novo* nonconvulsive epilepsy, it remains possible that TBI may worsen pre-existing epileptic or hyperexcitable states as our study suggests. Understanding TBI-induced exacerbation of nonconvulsive seizures requires knowledge of the mechanisms underlying the pre-existing condition. Fortunately, there is extensive information about the nature of SWDs in rodents. The SWDs and the accompanying freezing behavior have been studied in rodents as a model of absence epilepsy. These electrographic events occur spontaneously in a number of inbred rodent strains including the GAERS (Genetic Absence Epilepsy Rats from Strasbourg) and WAG/Rij (Wistar Albino Glaxo rats from Rijswijk) rats³⁰ as well as the Sprague-Dawley strain used in our experiments. Spontaneous SWDs with associated freezing behavior result from alterations in the excitability of cortical-thalamic circuits with particular involvement of the reticular nucleus of the thalamus. Studies identifying these circuits have been extensively reviewed.^{31,32}

Historically, there has been considerable debate about the focal origin of SWDs in rodents. Recent models, however, now suggest that a cortical focus of hyperexcitability is necessary for the expression of SWDs and that thalamic circuitry is secondary and serves to modify pattern of the discharges.³¹ This may explain why, in our study, mild TBI, which impacts primarily the cortex, provoked SWDs to the same degree as moderate or severe TBI. The SWDs were also exacerbated in other injury models that primarily affect the cortex including cortical impact injury²⁶ and cortical photothrombotic stroke.²⁵

Conclusions

This study found that fpTBI significantly increased expression of SWDs associated with behavioral freezing one year after injury. Increased expression of SWDs was independent of injury severity.

FIG. 6. Histological evaluations of brain damage (regional tissue volumes) one year after mild, moderate, or severe fluid percussion traumatic brain injury (TBI), sham-operated animals, and age-matched naïve animals. Selections show tissue volumes for cortex, external capsule, and hippocampus expressed as the ratio of the injured hemisphere volume (ipsilateral) to the noninjured (contralateral) hemisphere. ($n=4, 9, 10, 11$, and 6 for the naïve, sham, mild, moderate, and severe groups, respectively). *Analysis of variance group effect, $p < 0.05$, $p < 0.0001$, $p > 0.05$ for cortex, external capsule, hippocampus).

The SWDs and behavioral arrest such as those recorded in this study have been described as nonconvulsive seizures and have been used as models of absence seizures. It was not possible to determine whether increase expression of SWDs after TBI reflected acquired epilepsy or increased expression of existing pathology because naïve animals expressed SWDs without TBI. While the study of SWDs in rodents after TBI may not be an ideal model of epileptogenesis, such studies may be important investigations of the effects of TBI in brain with underlying epilepsy, because patients with absence epilepsy are at increased risk for TBI.³³

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Author Disclosure Statement

No competing financial interests exist.

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