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Characterization of neonatal seizures in an animal model of hypoxic-ischemic encephalopathy

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SUMMARY

Objective—In this study, we use time-locked video and electroencephalograph (EEG) recordings to characterize acute seizures and EEG abnormalities in an animal model that replicates many salient features of human neonatal hypoxic-ischemic encephalopathy (HIE) including the brain injury pattern and long-term neurologic outcome.

Methods—Hypoxia-ischemia (HI) was induced in 7-day-old rats by ligating the right carotid artery and exposing the pups to hypoxia for 2 hours (Rice-Vannucci method). To identify seizures and abnormal EEG activity, pups were monitored by video-EEG during hypoxia and at various time points after HI. Occurrence of electroclinical seizures, purely electrographic seizures and other abnormal discharges in the EEG were quantified manually. A power spectrum analysis was done to evaluate the effects of HI on EEG spectra in the 1 to 50 Hz frequency band.

Results—During hypoxia, all pups exhibit short duration, but frequent electroclinical seizures. Almost all pups continue to have seizures in the immediate period following termination of hypoxia. In over half of the HI rats seizures persisted for 24 hours, for some of them, the seizures continued for more than 48 hours. Seizures were not observed in any rats at 72 hours after HI-induction. A significant reduction in background EEG voltage in the cortex ipsilateral to the ligated carotid artery occurred in rats subjected to HI. In addition, purely electrographic seizures, spikes, sharp waves and brief runs of epileptiform discharges (BRED) were also observed in these rats.

Significance—HI-induction in P7 rats using the Rice-Vannucci method resulted in the development of seizures and EEG abnormalities similar to that seen in human neonates with HIE. Therefore, we conclude that this is a valid model to test the efficacy of novel interventions to treat neonatal seizures.

Keywords

Epilepsy; EEG; video; electroclinical; hypoxia; ischemia

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DISCLOSURE OF CONFLICTS OF INTEREST

None of the authors have any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

INTRODUCTION

Seizures occur more often in the neonatal period than at any other time of human life. The most common cause of neonatal seizures is hypoxic-ischemic encephalopathy (HIE),[1, 2] a serious condition with a suggested incidence of 1 to 8 cases per 1000 live births.[3] Hypoxia-ischemia (HI) in neonates also results in injury to various brain regions[4] and survivors of such injury can experience a multitude of neurological problems such as cerebral palsy, learning deficits and epilepsy.[2, 5, 6] Clinical as well as basic science research studies suggest that seizures may exacerbate HI-induced brain injury and contribute to poor neurological outcome[7–13] (but also see[14, 15]). Current antiepileptic drugs were developed using adult animal models and are not fully effective in treating neonatal seizures. Due to developmental differences, an immature brain may respond differently to an injury and a treatment than a mature brain. Therefore, to find the most effective treatment for a neonatal disease, it is imperative to test the efficacy of novel drugs in an animal model that not only accurately replicates the etiology and symptoms of a disease, but also the age of onset of the disease.

The Rice-Vannucci model of HI-induction in the postnatal day 7 (P7) rat[16] is widely used to study neonatal HIE. This model exhibits many of the salient features of human neonatal HIE such as the extent of brain injury,[17–19] development of epilepsy in some, but not all animals in later life,[20] and the learning and memory deficits.[21] However, a complete characterization of acute seizures in this model is still lacking. In the current study, we use a synchronized video and electroencephalograph (EEG) recording technique to study characteristics of HI-induced neonatal seizures that occur during and shortly following the time of insult. For certain disease conditions such as epilepsy, continuous video-EEG recording has become the gold standard to confirm the presence of convulsive seizures and also to detect non-convulsive or subclinical seizures. Video-EEG is particularly useful in the assessment of neonates in whom a large percentage of seizures are subclinical and for whom differentiation of normal movements from ictal motor activity can be difficult if not impossible based on behavioral observation alone. Further, in neonates, there is often dissociation between clinical (behavioral) seizures and EEG phenomena (known as electroclinical uncoupling), resulting in resolution of behavioral manifestations of seizures despite on-going electrographic seizures. Because of this phenomenon, it has been observed that some drugs effectively stop behavioral seizures in neonates without stopping electrographic seizures. Therefore, the use of video-EEG is being increasingly employed in clinical settings to identify and manage neonatal seizures. However, because of technical challenges, EEG is rarely obtained in animal models of neonatal diseases. In the current study, we describe the characteristics of acute electroclinical seizures and the background EEG in the Rice-Vannucci P7 rat model of neonatal HIE. This information will be helpful in assessing how closely this model replicates the human neonatal HIE condition as well as the model's validity for testing the efficacy of drugs used in the treatment of neonatal seizures.

METHODS

All animal procedures were performed according to the protocol approved by the Institutional Animal Care and Use Committee of the University of Colorado Anschutz

Medical Campus (UC-AMC). Also, all efforts were made to reduce animal suffering and the number of animals used. Timed pregnant Sprague-Dawley rats were obtained from Charles River Laboratories (Wilmington, MA). The pregnant rats were at the 14th day of gestation (E14) on arrival at the laboratory animal facility of the UC-AMC, and delivered the pups at E22 or E23. The litter size varied from 11 to 13 pups and the weights ranged between 12.5 to 15 grams at postnatal day 6 (P6). Only male pups were used in the current study. The experiments were performed in a sequence depicted in figure 1.

Electrode implantation

To record electrical activity of the brain, P6 rats (n = 16) were implanted bilaterally in the parietal cortex with silver electrodes (0.008″ outer diameter; A-M Systems, Carlsborg, WA). The holes for implantation were made 2.5 mm behind the bregma and 3 mm lateral from midline sutures. The length of the electrodes from the bottom of the pedestal was 3 mm. This length assured that the recording electrode was sufficiently inside the brain, but not too deep (to decrease cortical damage), to obtain good quality signal (supplemental figure 1). Two electrode placement schemes were employed, differing only in the placement of the reference electrode. In the first, a common reference electrode was placed near the lambda over the left hemisphere; in the second, the active electrode in each hemisphere was referenced to a separate electrode positioned near the lambda in the same hemisphere as itself. Except for the seizure characterization data, which were obtained from both configurations and are presented in the table 1, all data were acquired using the second configuration. The electrode assembly was fixed to the skull with tissue adhesive and dental acrylic cement. The entire implantation procedure was performed under isoflurane anesthesia (2–4% for induction, initiated at 2%; 1–1.5% for maintenance). After the surgery, the rats were treated with an analgesic (0.1 mg/kg buprenorphine) once every 12 hours for 48 hours.

Hypoxia-ischemia induction

HI was induced in P7 pups (n = 12) according to a published protocol.[16,20] The pups were anesthetized with isoflurane and, after infusion of Marcaine (0.5%) at the incision site, a small longitudinal cut was made along the midline of ventral cervical skin. The right common carotid artery was then identified and double ligated with 4-0 polyglycolic acid suture. The incision was closed with 4-0 nylon Dermalon sutures or with skin glue. The entire operation lasted for 10 to 12 minutes. Following carotid ligation, the pups remained with the dam in a warm cage for 60 to 105 minutes. The pups were then separated and monitored for 30 minutes by video-EEG, following which they were exposed to hypoxia for 2 hours in an airtight chamber that was filled with 8% oxygen and 92% nitrogen gas mixture. The oxygen content of the chamber was monitored using an oxygen sensor (Dräger Pac 7000, Pittsburg, PA) and was maintained between 8 and 8.3%. The temperature and humidity of the chamber were also tightly controlled and maintained at 36.5° Celsius and 60 to 70% respectively as variations in these parameters can affect both mortality rate and extent of the HI-induced injury.

Video-EEG recording

EEG signals synchronized with digital video were recorded using the Stellate Harmonie system (Natus Medical, San Carlos, CA). The EEG data were collected with a sampling rate of 1000 Hz and stored on a hard disk for off-line analysis. P6 pups (n = 12) were implanted with electrodes and, at P7, underwent 30 minutes of baseline video-EEG recording prior to carotid ligation. Following ligation and prior to hypoxia, a second 30-minute video-EEG record was acquired. The pups were continuously monitored by video-EEG during hypoxia. The video-EEG recording was continued for 2 more hours after completion of hypoxia. To find out if the pups experienced acute spontaneous seizures following HI-induction, they were monitored by video-EEG for 4 hours a day (two 2 hour sessions separated by a 2 hour break, during which the pups were housed with the dam) 24, 48 and 72 hours after HI-induction. A separate group of rats whose carotid artery was not ligated and who were not exposed to hypoxia (control rats, n = 4) was monitored using video-EEG under the same conditions and for the same duration as HI rats.

Seizure characterization

Video-EEG records were analyzed by the first author of the current paper (DS) and all the events (electroclinical and purely electrographic seizures) identified by DS were checked for accuracy by a board certified clinical epileptologist (AW) and a researcher with experience in animal EEG analyses (YR). Electroclinical seizures were defined by an EEG pattern that differed from background in either amplitude, frequency or both, evolved over time and contained spikes or sharps lasting for 10 seconds or more and were associated with a change in the rat's behavior. Electrographic seizures were defined as seizures observed in the EEG that were not associated with a behavioral correlate on video. Brief runs of epileptiform discharges (BRED) were defined as EEG patterns similar to an electrographic seizure with duration greater than 2 but less than 10 seconds. These might or might not have an associated change in the rat's behavior.

Power Spectrum Analysis

The power spectra were determined using a Fast Fourier Transform (FFT) algorithm written using Visual Basic (Microsoft, Redmond, WA) subroutines. A rectangular window was used with epoch sizes of 8192 points (sampling rate = 1000 Hz) and frequency bins of 1 Hz spanning 1 to 50 Hz. To compare groups of epochs characterizing different animal states (e.g., baseline, ictal during hypoxia, interictal during hypoxia, interictal post-HI background), we identified at least 10 EEG epochs (contiguous or not) that were acquired during that state and were devoid of movement artifacts. The average of the power for the different epochs in each frequency bin was calculated for each of the two groups (animal states) to be compared. To investigate the change in the shape of the power distribution, the actual power was normalized. To statistically compare groups, the power within the frequency bins was integrated into delta (1–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), beta (13–25 Hz), and gamma (25–50 Hz) bands. Comparisons were performed using methods described below.

Statistical Analysis

GraphPad Prism 5 statistical software (GraphPad Software Inc., San Diego, CA) was used for statistical analysis. Friedman test with Dunn's test for multiple comparisons was used to compare integrated power spectral values of EEG obtained up to 72 hours after carotid ligation with the corresponding pre-ligation baseline value for each rat, and to analyze age-dependent changes in EEG power in the control rats. An unpaired t-test was used to identify statistical differences in integrated power spectral values of EEG at P10 between control and HI rats. A one-way analysis of variance (ANOVA) with Tukey's post-hoc test was used to evaluate the effect of HI on spike or sharp wave activity. A p value of less than 0.05 was considered statistically significant.

RESULTS

Characteristics of hypoxia-ischemia induced seizures

Seizures were not observed in the control rats during any video-EEG recording sessions. In the HI group of rats, seizures were not detected during baseline EEG recording or in the period between carotid ligation and exposure to hypoxia. However, all pups quickly developed behavioral seizures with a clear electrographic correlate (electroclinical seizure) upon exposure to the hypoxic environment following carotid artery ligation (n = 12, table 1). The behavioral seizures consisted of clonic seizures, tonic posturing of the trunk, tonic-clonic seizures, facial twitching and stiffening of the tail (video 1, supplemental material). The clonic and tonic seizures could involve all extremities or originate unilaterally. Those which did originate unilaterally did frequently generalize. The EEG activity associated with the electroclinical seizures showed an evolution of amplitude, frequency or both and contained spikes and sharps (figure 2A). The rats experienced frequent, typically short duration seizures during hypoxia (table 1). Two out of twelve pups (16%) also developed purely electrographic seizures during hypoxia at an average frequency of 1 seizure per hour (table 1).

In the period immediately following hypoxia i.e., during the reperfusion period, 11 out of 12 rats (91%) continued to have electroclinical seizures. These seizures, similar to seizures of the hypoxic period, were brief and frequent (table 1). The behavioral seizures, which correlated with a change in EEG activity, consisted of clonic, tonic and tonic-clonic seizures (figure 2B). Some of the rats (3/12) also developed purely electrographic seizures (table 1). Twenty-four hours after the initial insult, 66% of the rats (8/12) continued to exhibit electroclinical seizures (table 1). For these rats, both the seizure frequency and the total time seizing were lower than during the hypoxic and the reperfusion period (table 1). The behavioral seizures consisted of body jerks associated with tonic and clonic seizures (figure 2C). Many of these rats (7/12) also manifested purely electrographic seizures (table 1). Only 25% of HI rats (3/12) continued to have electroclinical seizures 48 hours following HI-induction (table 1). Neither electroclinical nor purely electrographic seizures were observed in any rats 72 hours after HI-induction (n = 12, table 1).

To examine whether there was any change in the shape of the EEG power spectrum within the 1 to 50 Hz frequency band during the electroclinical seizures, the sum of the raw EEG

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