Epileptogenic Effect of Hypoxia in the Immature Rodent Brain

F. E. Jensen, MD,* C. D. Applegate, PhD,† D. Holtzman, MD, PhD,* T. R. Belin, MS,‡ and J. L. Burchfiel, PhD†

The response to cerebral hypoxia/ischemia may be different in the neonate compared to other age groups. An in vivo model was developed in the rat to determine whether there are age-dependent differences in the effects of hypoxia on electroencephalographic (EEG) activity. EEG recordings were obtained from Long Evans hooded rats deprived of oxygen at five ages: postnatal days 5 to 7, 10 to 12, 15 to 17, 25 to 27, and 50 to 60. Oxygen concentration was varied from 0, 2, 3, and 4% between animals. EEGs were recorded in all animals before, during, and at 1 hour after exposure to the hypoxic condition and at 1 to 7 days afterward in a subset of animals. All animals were deprived of oxygen until the onset of apnea and bradycardia to 20 to 40% of baseline heart rate values. Hypoxia resulted in isoelectric EEG significantly more frequently in the animals deprived of oxygen at postnatal days 25 to 27 and 50 to 60 than in the younger age groups. A highly significant effect was that the animals deprived at postnatal days 5 to 17 revealed a high incidence of epileptiform EEG activity during hypoxia. In contrast, the older animals exhibited only rare isolated EEG spikes before reaching an isoelectric EEG. The severity of hypoxia-induced epileptiform EEG changes was highest in the animals subjected to moderately hypoxic conditions (3% and 4% oxygen) at postnatal days 10 to 12. Furthermore, epileptiform changes persisted for hours to days following prolonged episodes of hypoxia in the younger animals. This study demonstrates a unique response of the immature brain to exhibit epileptiform activity during hypoxia.

Jensen FE, Applegate CD, Holtzman D, Belin TR, Burchfiel JL. Epileptogenic effect of hypoxia in the immature rodent brain. Ann Neurol 1991;29:629–637

Global cerebral hypoxia/ischemia in the perinatal period may result in an increased risk of cerebral palsy [1] as well as seizure disorders and developmental delay [2, 3]. The perinatal period has also been thought to be a period of increased seizure susceptibility from various etiologies [4, 5]. Human infants are exposed to hypoxia or decreased cerebral blood flow in a variety of clinical settings including perinatal asphyxia, respiratory arrest, near-miss sudden infant death syndrome, and procedures such as extracorporeal membrane oxygenation (ECMO) and hypothermic cardiopulmonary bypass for correction of congenital heart disease. Therefore, it is important to understand the pathogenesis of cell injury in the hypoxic immature brain and to identify physiological indicators of hypoxic/ischemic injury.

Electroencephalographic (EEG) activity is an important modality for monitoring brain function in human neonates. We developed an in vivo rodent model of global central nervous system hypoxia that is suitable for the study in EEG responses in rats at intervals from postnatal day (P) 5 to adulthood. Using this model, we measured EEG and electrocardiographic (ECG) responses to varying degrees of hypoxia at different stages of brain maturation.

Materials and Methods

Subjects

Long Evans rats were exposed to hypoxic or anoxic gas mixture at 5 ages: P5 to 7, P10 to 12, P15 to 17, P25 to 27, and P50 to 60 (adults). A total of 119 rats were studied. A separate group of animals were used to obtain arterial blood gases during normoxia versus hypoxia, because sampling of even 1 or 2 ml from the young animals depletes a significant fraction of their blood volume and could result in ischemia. For those measurements, 14 rats at age P10 and 4 adult rats were used.

Electrode Implantation and EEG Recording

Electrode plugs (Samtec, New Albany, IN) were implanted epidurally with ether anesthesia for rats at P5 to 27 and pentobarbital sodium (Nembutal) (55 mg/kg intraperitoneally) for adult rats. A short-acting anesthetic was adequate for the young rats because the surgical procedure only lasted 5 to 7 minutes, whereas electrode placement took 30 to 40

From the *Department of Neurology, Children's Hospital and Harvard Medical School, Boston; †Department of Neurology, University of Rochester Medical Center, Rochester, NY; and ‡Department of Statistics, Harvard University, Cambridge, MA.

Received Jul 31, 1990, and in revised form Nov 30. Accepted for publication Dec 16, 1990.

Address correspondence to Dr Jensen, Department of Neurology, Enders 2, Children's Hospital, 300 Longwood Ave., Boston, MA 02115.

minutes in the adults, requiring pentobarbital sodium anesthesia. Different electrode arrays were used for different ages. In rats at P5 to 27, two electrodes were placed through burr holes 1.5 mm on either side of the sagittal suture midway between the bregma and lamda sutures. For adult animals, two electrodes were placed under stereotaxic control −1.0 mm and −6.0 mm posterior, 3.75 mm lateral from bregma over each hemisphere. Electrode plugs were mounted on the skull with rapid-acting adhesive (Pella, Redding, CA) and dental acrylic (Lang, Chicago, IL). In the young animals, EEG responses and behavior were fully recovered by 90 minutes following ether anesthesia, and they were studied between 2 and 24 hours after surgery. Full EEG and behavioral recovery was evident in the adults about 6 hours following anesthesia; these animals were studied 24 to 48 hours after surgery. EEG activity was recorded on a Grass model 6 polygraph (Grass Instruments, Quincy, MA) in a bipolar mode using pairs of electrodes. Specific electrode montages varied depending on the age of the animal, with interhemispheric recordings made in all rats, and additional intrahemispheric recording made in the adult rats. ECG was recorded from two subcutaneous needle electrodes placed on either side of the dorsal trunk. Baseline EEG and ECG activities were recorded from all animals before exposure to hypoxic conditions. EEG was continuously monitored throughout the period of hypoxia. Behavior was noted during EEG recording. EEG and ECG were continuously monitored for 1 hour after oxygen deprivation. A subset of animals in each age group had EEG recordings at intervals up to 7 days after hypoxia.

Exposure to Hypoxic or Anoxic Conditions

Animals at all ages were randomized for exposure to either 0, 2, 3 or 4% oxygen (O_2). Animals were lightly restrained on all four limbs. Nitrogen (N_2) gas was infused into the chamber to obtain the desired reduction in O_2 concentration. An O_2 meter (Instrumentation Laboratories, Lexington, MA) within the chamber was used to monitor the O_2 concentration during infusion of N_2. Body temperature was maintained within 2°C of baseline by placing the animal next to a plastic bag filled with water at 36°C. In order to normalize exposure time to the hypoxic condition across age groups, animals were maintained at a given O_2 concentration until they became apneic and heart rate was reduced to a rate less than 40% of baseline value for 60 seconds. At this point, most animals could be resuscitated easily with room air. This ensured that each age group received the maximal duration of hypoxia that was compatible with survival. After a 1-hour recovery period during which EEG recordings were made, animals were returned to their cages.

Analysis of EEG and ECG Data

EEG and ECG tracings were reviewed by observers who did not know the degree of hypoxia and age of the animal. The EEG during exposure to the hypoxic condition and recovery was analyzed for the presence of isoelectric and epileptiform EEG changes and for the latency to the first event of either type. For each animal, the EEG during exposure to the acute hypoxic condition was compared to its own prehypoxic baseline EEG. EEG recordings from age-matched control littermates not exposed to the hypoxic condition were used for comparison during the recovery period. Heart rate was measured before hypoxia (baseline), at the onset of each identified EEG or behavioral change, and at the end of the hypoxic period prior to resuscitation.

Arterial Blood Gas and pH Measurements

In order to evaluate whether the degree of hypoxemia differed between young and adult rats, we obtained arterial blood samples from animals at P10 to P60 during exposure to 3% O_2. Prior to exposure, EEG and ECG electrodes were implanted as described above, and adult animals underwent femoral artery cannulation while anesthetized. Baseline EEG and ECG recordings were obtained from all animals 3 to 4 hours after recovery from anesthesia. Because the blood volume of a rat at P10 is small, it was necessary to use separate control and hypoxic animals. Arterial samples were obtained from the left ventricle in the P10 animals by direct puncture with a 27-gauge needle through the left anterolateral aspect of the chest wall anesthetized with 2% lidocaine (Xylocaine). Control arterial blood samples were obtained from P10 animals in room air and from adults immediately prior to undergoing hypoxia. EEG and ECG activities were monitored during exposure to hypoxia. Hypoxic samples were obtained from the P10 animals at the onset of the first epileptiform EEG and behavioral change. In the adult animals, a single arterial sample was obtained at the onset of isoelectric EEG activity. The total time of the hypoxic period before sampling was noted for each animal. All hypoxic samples were obtained while the animals were continuing to breathe 3% O_2. Arterial samples were placed on ice and analyzed by a Corning blood gas analyzer to obtain O_2 pressure (Po_2), carbon dioxide pressure (Pco_2), O_2 saturation, and pH.

Statistical Analysis

The data were considered as consisting of independent variables (O_2 and age) measured on a continuous scale and the dependent variables as binary (presence of isoelectric or epileptiform EEG change), ordinal (degree of epileptiform EEG change), and continuous (heart rate). To evaluate the dependence of binary outcome on the predictors, a sequence of logistic regression models was fit to the data [6]. Tests of significance were based on changes in the likelihood ratio statistic between nested models and referred to a chi-square distribution. To assess whether an interaction exists between the presence of an epileptiform response and an isoelectric response, a sequence of log-linear models was fit to the four-way table of (age) × (O_2) × (epileptiform response) × (isoelectric response). A term involving the cross product of the 2 × 2 table of (epileptiform) × (isoelectric) response models the dependence between the two response variables. By fitting a log linear model [7] to the four-way table, the significance of the dependence between response variables could be assessed after controlling for age and O_2. A two sample t statistic was used to assess differences between average reduction in heart rate and blood gas measures at the time of an isoelectric EEG versus an epileptiform change in the EEG. Computations were carried out using the CATMOD procedure in the SAS software package (SAS Institute, Cary, NC, 1985).

Results

Prehypoxic EEG Characteristics

Qualitative analysis of baseline EEG activity at different ages revealed a progressive maturation from a predominantly discontinuous pattern to a predominantly continuous one. At P5 to 7, background activity consisted of very low amplitude (< 5 μV), slow (1- to 3-Hz) activity interrupted at irregular intervals by higher voltage, 3- to 6-Hz bursts (Fig 1A). By P10 to 12, the background activity consisted of higher amplitude baseline activity (8 to 12 Hz) more regularly punctuated by high, 3- to 6-Hz bursts (Fig 1B). High-voltage bursts were infrequent by P15. Background activity increased in frequency and amplitude to adult values between P15 to 17 and P25 to 27 (Figs 1C and D). The EEG frequency at P25 to 27 closely resembled the adult (6 to 12 Hz), except with a slightly lower amplitude. No spontaneous spikes or rhythmic spike/wave discharges were observed in nonhypoxic animals at any age.

EEG and Behavioral Characteristics during Acute O_2 Deprivation

Analyses were performed to evaluate the effect of age or O_2 concentration, independently, on EEG pattern and clinical behavior during acute O_2 deprivation. The prehypoxic behavior and EEG tracings served as a within-animal control for the acute EEG changes during hypoxia. A preliminary analysis identified the following categories of EEG patterns and associated behavior: (1) isoelectric EEG (electrocortical silence) with no observable movement of the animal except respirations (Fig 2E), (2) baseline activity with no epileptiform behavioral or EEG changes, (3) isolated EEG spike discharges without behavioral changes (Fig 2D), (4) isolated spike discharges associated with a behavioral change (myoclonic jerks of trunk or single limb, automatisms, and chewing) (Fig 2A), and (5) trains of EEG spike/wave discharges associated with behavioral change (usually tonic-clonic movement of head and limbs) (Fig 2B).

INDUCTION OF ISOELECTRIC EEG. O_2 deprivation resulted in the development of an isoelectric EEG in animals of all ages, but was most frequently seen in the adult group (Table 1; see Fig 2E). All adult animals exposed to 3% O_2 or less, and 3 out of the 4 adults exposed to 4% O_2 exhibited isoelectric EEG. When O_2 concentration was controlled for, the isoelectric EEG was seen significantly less frequently in the three younger age groups than in the P25 to 27 and adult rats ($p < 0.002$) (see Table 1). Ninety-five percent of the P25 to 27 animals, developed an isoelectric EEG during O_2 deprivation. Isoelectric EEG activity was not observed in the P5 to 7 animals exposed to 4% O_2, and only approximately 50% of the P10 to 12 animals

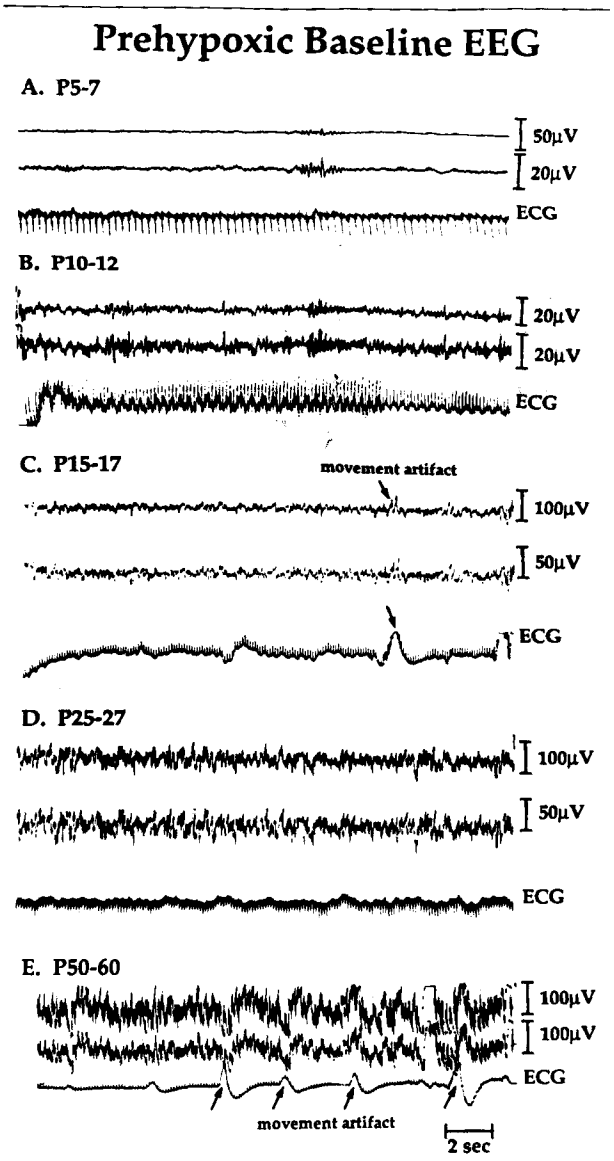

Fig 1. Baseline EEGs recorded before hypoxia from representative animals within each of the five age groups. (A) EEG of rats at postnatal days (P) 5 to 7 shows low-amplitude baseline punctuated by bursts of higher voltage slowing. (B) EEG in rats at P10 to 12 reveals increased baseline activity compared to P5 to 7 animals, but still is interrupted by bursts of high-voltage activity. (C through E) A progressive increase in amplitude and frequency is seen between P15 and P50. For each age, two channels of EEG activity are displayed. ECG activity is shown below the EEG tracings. See methods for details of electrode placement. Movement artifact is labeled where present in both EEG and ECG tracings.

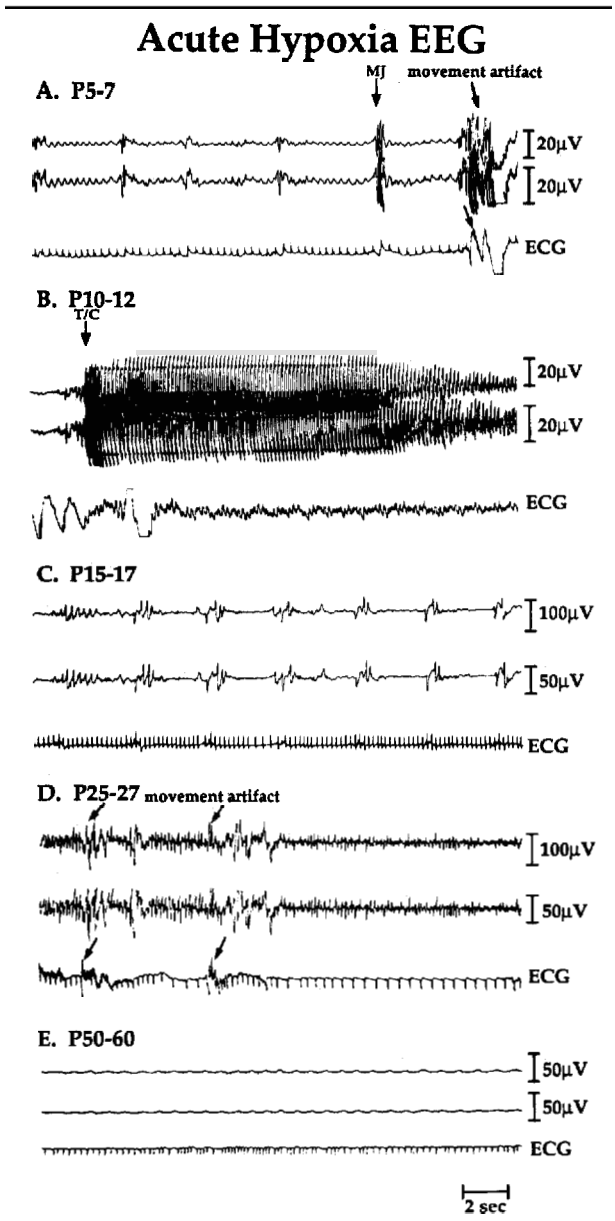

Fig 2. EEG activity during exposure to 3% O_2 in representative animals from each age group. (A) Spike discharges are seen in isolation, and in association with a myoclonic jerk (MJ) *in this rat at postnatal days (P) 5 to 7. (B) Tracing from a P10 to 12 rat showing train of spike discharges in association with tonic-clonic head and trunk activity* (T/C). *(C) Rhythmic spike discharges are seen in this rat from the P15 to 17 group, without any associated behavioral changes. (D) Low-amplitude spike activity seen in this rat from the P25 to 27 group, without any behavioral correlation. (E) An adult (P50 to 60) rat exhibits isoelectric EEG during O_2 deprivation. For each age, two channels of EEG activity are displayed. ECG activity is shown below the EEG tracings. See methods for details of electrode placement. Movement artifact is labeled where present in both EEG and ECG tracings.*

Table 1. Presence of the Development of Isoelectric EEG within Each Age Group and O_2 Concentration[a]

	Age				
O_2%	P5–7	P10–12	P15–17	P25–27	P50–60
0%	4/6	6/6	4/4	5/5	6/6
2%	4/8	5/5	4/4	5/5	4/4
3%	1/6	11/21	4/6	5/5	7/7
4%	0/4	2/4	1/4	5/5	3/4

[a]No. of animals in each group with isoelectric EEG/total no. in group.

eventually reached isoelectric state with moderate O_2 deprivation (3% and 4% O_2). With more severe O_2 deprivation (0% and 2%), the younger animals developed isoelectric EEGs at a frequency approaching that seen in the P25 to 27 and adult animals. Differences were observed in latency to onset of isoelectric EEG across all O_2 conditions. The longest latency (mean ± standard error of mean [SEM]) was seen in the P5 to 7 group (388 seconds ± 124 seconds), and decreased with age, with the shortest mean latency in the adults (167 seconds ± 19 seconds).

When age was controlled for, a significantly higher rate of isoelectric EEG events was present during anoxia than at 3% and 4% O_2 concentrations ($p < 0.003$ and $p < 0.004$, respectively). The frequency of isoelectric events was not significantly different during anoxia (0% O_2) and 2% O_2 across ages.

INDUCTION OF EPILEPTIFORM EEG CHANGE. Electrographic seizures were recorded frequently in the immature animals during the hypoxic period. The probability of an epileptiform event was (1) higher among younger animals, (2) higher with increasing oxygen content, and (3) more frequently seen in animals exposed to hypoxic compared to anoxic conditions.

Epileptiform EEG changes were pronounced in animals 5 to 17 days old during exposure to 3% and 4% O_2. At these ages, 80 to 100% of the animals exhibited epileptiform EEG changes (Table 2; see Figs 2A, B, and C). Controlling for O_2 concentration, the incidence of epileptiform change was significantly greater in the three younger groups of animals (P5 to 7, 10 to 12, and 15 to 17) than in the two older groups (P25 to 27 and 50 to 60) ($p < 0.0001$). Half of the P25 to 27 animals exhibited spike discharges without a behavioral correlate with exposure to 3% and 4% O_2 (see Fig 2D). The predominant response in the adults with exposure to any of the levels of O_2 was an isoelectric EEG (see Fig 2E). Only 2 of 7 adults exhibited epileptiform EEG activity before achieving an isoelectric EEG during exposure to 3% O_2 (see Table 2). These events consisted of isolated spikes without a behavioral change.

Table 2. Presence of Any Epileptiform EEG Change during Hypoxia at Each O_2 Concentration, and Presence of Epileptiform Change Associated with Tonic-Clonic Behavior at Each Across O_2 Concentrations

	Age				
	P5–7	P10–12	P15–17	P25–27	P50–60
O_2%[a]					
0%	3/3	1/6	2/4	1/5	0/6
2%	4/8	2/5	3/4	1/5	0/4
3%	5/6	18/21	6/6	2/5	2/7
4%	4/4	4/4	2/4	3/5	0/4
Frequency of tonic-clonic activity[b]	3/13	16/25	7/13	0/7	0/2

[a]No. of animals in each group with epileptiform EEG changes/total no. in group.
[b]No. of animals with epileptiform EEG change and tonic-clonic behavior/no. of animals with any epileptiform EEG change.

Controlling for the effect of age, anoxia was associated with a significantly lower rate of epileptiform events than 3% and 4% O_2 conditions ($p < 0.0002$ and $p < 0.007$, respectively).

In the P10 to 17 animals, a 1% change (from 2% to 3% O_2) altered the response from being predominantly isoelectric to epileptiform. In contrast, the responses of the other ages were more homogeneous across O_2 concentrations, with the younger animals rarely reaching isoelectric state at any O_2 concentrations, and animals at P25 and older rarely revealing epileptiform changes even at higher O_2 concentrations. The responses of the P10 to 17 animals appeared more similar to the younger animals at higher O_2 concentrations (3% and 4% O_2) and more closely resembled the adult pattern of response at lower O_2 concentrations (0% and 2% O_2).

BEHAVIORAL MANIFESTATIONS OF EPILEPTIFORM EEG RESPONSES. Trains of spike/wave discharges associated with tonic-clonic behavior were seen most frequently in the P10 to 12 and P15 to 17 animals, with 64% and 62% of these animals, respectively, exhibiting such responses (see Table 2). Only 19% of the rats at P5 to 7 exhibited sustained tonic-clonic activity. The most common response in the P5 to 7 group was myoclonus or automatisms (i.e., chewing or rearing) accompanying EEG spike discharges. The most common response in the P25 to 27 and adult rats was progression to isoelectric EEG, with no tonic or clonic activity seen under any of the conditions used.

INTERACTION BETWEEN PRESENCE OF EPILEPTIFORM EEG RESPONSE AND ISOELECTRIC EEG. Based on a log-linear model of bivariate binary response [7] and controlling for age and O_2 concentration, no significant interaction was found between epileptiform response and development of isoelectric EEG during hypoxia. Substantial interdependence between isoelectric and epileptiform EEG is ruled out, but the statistical analysis could not rule out a small interaction, due to the limited size of the sample. Hence, no further distinctions were made in our analyses between animals with de novo versus those with postictal isoelectric EEG. Epileptiform EEG activity was never preceded by an isoelectric EEG state.

ECG Responses

Heart rate was measured for each animal at the onset of an epileptiform event or isoelectric EEG. Under most O_2 conditions, younger animals were able to tolerate longer periods of hypoxia than older animals before becoming apneic and reaching 20 to 40% of their prehypoxia heart rate. Across ages and O_2 concentrations, the mean heart rate was 32 ± 16% (± standard deviation [SD]) of prehypoxic value at onset of isoelectric EEG. This rate was significantly lower than the heart rate seen at the onset of an epileptiform EEG change, the mean value of which was 52 ± 25% (± SD) ($p < 0.0001$). All animals showed some degree of bradycardia at onset of epileptiform EEG changes. No significant correlation could be found between type of epileptiform EEG response and heart rate during O_2 deprivation.

Arterial Blood Measurements before and during Hypoxia

Mean arterial blood gas and pH measurements under normoxic conditions were similar for the P10 and adult animals (Table 3). However, onset of epileptiform activity in P10 animals occurred under conditions of more moderate hypoxemia ($p < 0.0003$) than at the onset of isoelectric EEG in the adults (see Table 3). In addition, the P10 animals, but not the adults, became significantly acidotic at the time of sampling compared to their controls ($p < 0.0004$). No significant differences in P_{CO_2} were noted, as these values appeared highly variable even in the control animals. The mean duration of hypoxia at blood sampling was comparable in the two age groups, at 260 ± 172 seconds (± SD) for the P10 animals, and 330 ± 205 seconds for the adult group. Although the adult group was more severely hypoxemic at the time of sampling, no epilepti-

Table 3. Arterial Blood Measurements under Normoxic and Hypoxic (3% O_2) Conditions in P10 and Adult Rats[a]

	Po_2 (mm Hg)	Pco_2 (mm Hg)	O_2 Saturation (%)	pH
P10				
Normoxic control (n = 8)	93.1 ± 6.1	33.1 ± 6.7	97.2 ± 5.2	7.42 ± 0.05
Hypoxic (n = 6)	40.28 ± 6.2	27.9 ± 6.4	62.3 ± 11.9	7.16 ± 0.09
Adult				
Normoxic control (n = 4)	93.3 ± 10.9	34.2 ± 9.7	97.3 ± 0.6	7.43 ± 0.03
Hypoxic (n = 4)	12.33 ± 6.8	20.7 ± 7.7	13.8 ± 10.9	7.30 ± 0.13

[a]Mean values ± standard deviation.

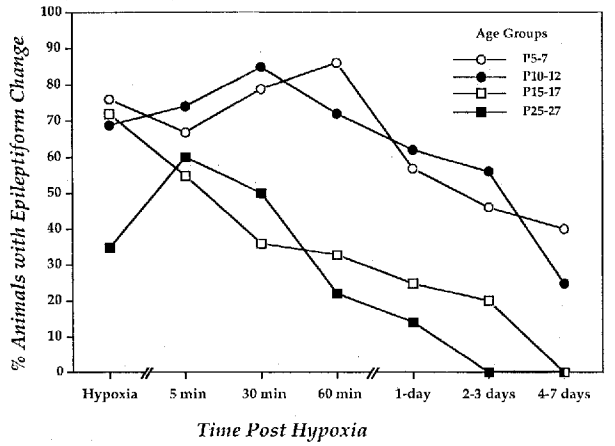

Fig 3. Frequency of epileptiform EEG changes in animals from four age groups sampled at 5, 30, and 60 minutes, and 1 to 7 days following O_2 deprivation. Data are represented as frequency of epileptiform change across all levels of O_2 exposure. P = age in postnatal days.

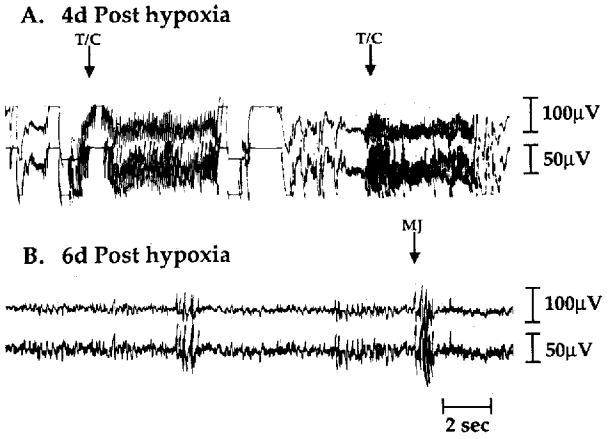

Fig 4. Example of EEGs from a rat exposed to 3% O_2 at 10 days of age. (A) Four days after hypoxia, showing trains of spike discharges associated with tonic-clonic head and trunk movement (T/C). *(B) Six days after hypoxia, with spike discharges associated with myoclonic jerks* (MJ). *See methods for details of electrode placement.*

form EEG or behavioral activity was seen prior to the onset of isoelectric EEG.

Posthypoxic EEG Characteristics

The number of animals surviving O_2 deprivation varied with age and O_2 concentration. All of the P5 to 7 pups survived all four levels of O_2 deprivation. The survival rate decreased with age, with 1 P10 to 12 animal dying during exposure to 3% O_2, and with 73% of the adult animals dying during exposure to 2, 3, or 4% O_2. Under anoxic (0%) conditions, none of the P5 to 7 or P10 to 12 rats died, but 75% of the P15 to 17 and P25 to 27 animals and all of the adults died.

Within the first 1 hour following return to room air, EEG activity was slower than the prehypoxia activity in both adult and immature rats after all levels of O_2 deprivation. Epileptiform EEG changes were present in up to 86% of P5 to 7 rats and 85% of P10 to 12 rats during the first 1 hour after hypoxia, compared to 55% and 60% of the P15 to 17 and P25 to 27 groups, respectively (Fig 3). Four of the 7 P5 to 7 animals and 11 of 11 P10 to 12 animals that had not shown any epileptiform EEG changes during acute hypoxia had spike activity with or without behavioral events in the first hour of recovery. In the P15 to 17 and P25 to 27 groups, only 2 of 5 and 3 of 13 animals exhibited such epileptiform changes in the 1 hour after hypoxia.

Epileptiform EEG changes were present in some animals for several days following hypoxia (see Fig 3; Fig 4). During the 1 to 7 days that animals were followed after hypoxia, random EEG sampling revealed an apparent dropoff in epileptiform events over time in all groups. The two younger groups consistently exhibited more changes than the two older groups. At 7 days, 2 of 5 P5 to 7 and 4 of 16 P10 to 12 rats tested showed epileptiform EEG abnormalities.

Discussion

These results demonstrate an effect of both age and O_2 concentration on the response to hypoxia in the rat. They also demonstrate the unexpected finding that hypoxia is acutely epileptogenic in immature animals, but not in adults. This effect is strongest between 10 to 17 days of age. Animals in this age group appear to be most sensitive to changes in O_2 concentration, since

only a 1% change in O_2 results in the predominant response switching from epileptiform to isoelectric EEG. In general, the degree of O_2 deprivation also affects outcome, with isoelectric EEG occurring more often under anoxic and severe hypoxic (2% O_2) conditions, and epileptiform EEG changes being more frequent under conditions of moderate hypoxia (3 and 4% O_2). As age increases, moderate hypoxia induces changes more characteristic of the response of the young animals to anoxia, indicating a potential interaction between age and O_2 concentration on EEG activity.

Baseline EEG recordings reveal prominent maturational changes taking place between P5 and P25. The pattern of increasing baseline organization with age is similar to patterns observed in other animal species [8]. The discontinuous EEG pattern seen at P5 to 7 and P10 to 12 in the rat is also a characteristic of human preterm and term infants. In addition, epileptiform EEG changes were rarely associated with tonic-clonic behavior in the youngest animals in our study (P5 to 7), but were more often associated with automatisms and myoclonic events, similar to epileptiform behavior in human neonates. Baseline EEG characteristics may be a predictor of response to hypoxia, an association that must be tested in other species including the human.

Both epileptiform and isoelectric EEG changes occur in association with relative bradycardia. Although no cerebral blood flow measurements were performed, these events occurred when heart rate was reduced to at least 60% of baseline, suggesting that ischemia might have accompanied hypoxia. Marked decreases in heart rate were associated with isoelectric EEG, while epileptiform EEG activity was associated with more modest reductions in heart rate. Direct measurement of cerebral oxygenation, glucose utilization, and blood flow will be necessary to fully determine whether the bradycardia actually is associated with cerebral ischemia. While these measurements were beyond the scope of this study, we did evaluate arterial blood gases as a measure of the degree of hypoxemia present during 3% O_2 deprivation in P10 and adult rats, as these ages showed significantly different EEG responses. It appears that hypoxia-induced epileptiform changes occur in the P10 animals in association with moderate decreases in Po_2 and more severe decreases in pH. Approximately the same duration of hypoxia results in significantly lower Po_2 values and isoelectric EEG states in the adults. However, the adult animals did not display any epileptiform changes prior to hypoxia, when their Po_2 values were higher and presumably at similar levels to those associated with epileptiform activity in the P10 animals. This would suggest that the epileptiform changes induced by hypoxia are determined by maturational factors, rather than by degree of hypoxemia alone. Finally, because most of these changes occur relatively early in hypoxia, they are not likely to be affected by differences in the total duration of hypoxia.

The ages at which hypoxia frequently induces epileptiform EEG activity (P5 to 17) correspond to a period of development during which many neurochemical and electrophysiological changes are known to be taking place. Although the immature brain has previously been thought to be less vulnerable to the effects of hypoxia and ischemia than the adult brain [9, 10], recent studies suggest that the immature brain may actually be more sensitive than the adult to neuronal injury induced by activity at the *N*-methyl-D-aspartate (NMDA) excitatory amino acid receptor subtype [11]. The NMDA receptor is thought to mediate hypoxic/ischemic cell death [12, 13] and has also been implicated in the generation of seizure activity [14]. NMDA toxicity has been shown to peak between P6 and P14 in the rat [11], a time frame similar to that during which hypoxia-induced epileptiform EEG activity is most pronounced. Because of its epileptogenic properties, the NMDA receptor may be involved in the epileptiform changes seen in the hypoxic younger animals in the present study. In many areas of the brain, the density of the NMDA receptor type peaks above adult values within the first 3 weeks of life [15]. Another glutamate receptor subtype thought to be involved in epileptogenesis, the quisqualate receptor, also exhibits a maturational peak before the third week of life in the rat [15, 16]. Another factor that might make the immature brain more susceptible to seizure activity from any cause is that the major inhibitory neurotransmitter, gamma-aminobutyric acid (GABA), and its receptors do not reach adult levels until the third or fourth week of life in the rat [17–19].

Electrophysiological evidence confirms that the immature nervous system is more excitable than adult tissue. Immature hippocampal slices maintained in vitro exhibit spontaneous seizures [20–23]. IPSPs are poorly developed in slices from animals 1 week old or less [19, 23, 24]. While excitatory amino acid neurotransmission is predominating over GABAergic transmission during the developmental window in which we demonstrate a predilection of hypoxia-induced epileptiform change, other factors could cause further neuronal depolarization. In the rabbit, the maturation of NA^+/K^+-ATPase is not completed until after the first 2 weeks of life. Low concentrations of Na^+/K^+-ATPase are associated with local increases in $[K^+]_o$ even under normoxic conditions, leading to excitatory phenomenon such as spreading depression [25]. Furthermore, $[K^+]_o$ has been shown to rise rapidly in response to O_2 deprivation in adult rat brain, but more gradually in animals between 4 and 14 days [26]. This gradual rate of rise may allow for enhanced excitability,

rather than the rapid onset of isoelectric EEG that is seen in the adult. Cytoplasmic calcium-binding proteins appear to be at lower levels than in the adult [27], potentially making immature neurons less able to buffer large amounts of Ca^{++} under depolarizing and hypoxic conditions.

Thus, the seizure activity demonstrated in young animals at P5 to 17 during O_2 deprivation may be multifactorial in nature. It will be necessary to correlate more precisely the neurochemical and electrophysiological changes with the sensitivity to hypoxia-induced seizure activity.

While we demonstrate that hypoxia is epileptogenic during an early period of development in the rat, it is not yet known whether the epileptogenic activity contributes to the increased pathology that has also been observed at this age in response to hypoxia/ischemia [11]. It is also unclear how the developmental period between P5 and 17 in the rat relates to human neonatal development. However, the bursts of high-voltage activity that interrupt the baseline activity in the P5 to 7 and P10 to 12 groups appear to be similar in nature to those seen in human preterm and term infants.

An important observation is that the epileptogenic effect of hypoxia is not restricted to the acute hypoxic period. In some of the P5 to 17 animals we observed EEG seizure activity persisting for hours to days. Those ages showing the most severe epileptiform EEG changes during hypoxia revealed similar changes up to 7 days after hypoxia. The long-term consequences and etiology of hypoxia-induced seizures in the perinatal period are under active investigation in our laboratory. Preliminary results reveal an increased susceptibility to pentylenetetrazol-induced seizure activity in adult animals exposed to 3% O_2 at 10 days of age [28]. It is not yet clear whether this persistent effect is related directly to hypoxia or is a function of the seizure activity itself. Pharmacological blockade of acute seizure activity during hypoxia may elucidate this mechanism. The extent to which the posthypoxic seizure activity contributed to the long-term changes in seizure susceptibility remains to be determined. This posthypoxic period is clinically relevant because it can easily be monitored by EEG and because it represents a potential therapeutic window for the prevention of later seizure activity.

These results were presented in part and in preliminary form elsewhere [29].

This research was supported by National Institutes of Health-National Institute of Child Health and Human Development (NICHD) grant HD00807 (F.E.J.) and in part by the Kaplan Foundation and Mental Retardation Research Center grant P30-HD18655 from NICHD.

The authors wish to thank Maria Chansky for technical assistance throughout the project. We thank Drs Cesare Lombroso and Antonio Lorenzo for most helpful discussions and advice during this project.

References

1. Nelson KB, Ellenberg JH. Antecedents of cerebral palsy. Multivariate analysis of risk. N Engl J Med 1986;315:81–86
2. Brown JK. Infants damaged during birth: perinatal asphyxia. In: Hull D, ed. Recent Advances in Pediatrics. London: Churchill Livingstone, 1976:57–88
3. Lombroso CT. Etiological and preventive aspects of epilepsy in the developing nervous system. Epilepsia 1987;28(suppl):25–35
4. Aicardi J, and Chevrie JJ. Convulsive status epilepticus in infants and children. A study of 239 cases. Epilepsia 1970;11:187–197
5. Moshe SL, Albala BJ. Maturational changes in postictal refractoriness and seizure susceptibility in developing rats. Ann Neurol 1983;13:552–557
6. Cox DR, Snell EJ. Analysis of binary data. New York: Chapman and Hall, 1989
7. McCallugh P, Nelder JA. Generalized linear models. New York: Chapman and Hall, 1983:145
8. Himwich W. Developmental neurobiology. Springfield, IL: Charles C Thomas, 1970
9. Duffy TE, Kohle SJ, Vanucci RC. Carbohydrate and energy metabolism in the perinatal rat brain: relation to survival in anoxia. J Neurochem 1975;24:271–276
10. Glass HG, Snyder FF, Webster E. The rate of decline and resistance to anoxia of rabbits, dogs, and guinea pigs from the onset of viability to adult life. Am J Physiol 1944;140:609–615
11. Ikonomidou C, Mosinger JL, Shahid Salles K, et al. Sensitivity of the developing rat brain to hypobaric/ischemic damage parallels sensitivity to N-methyl-aspartate toxicity. J Neurosci 1989; 9(8):2809–2818
12. Rothman SM, Olney JW. Glutamate and the pathophysiology of hypoxic-ischemic brain damage. Ann Neurol 1986;19:105–111
13. Rothman SM, Olney JW. Excitotoxicity and the NMDA receptor. Trends Neurosci 1987;10:299–302
14. Thompson AM, West DC. N-methyl-D-aspartate receptors mediate epileptiform activity evoked in some, but not all, conditions in rat neocortical slices. Neuroscience 1986;19:1161–1177
15. Insel TR, Miller L, Gelhard RE. The ontogeny of excitatory amino acid in the rat forebrain I: N-methyl-D-aspartate and quisqualate receptors. Neuroscience 1990;35:31–43
16. Trescher WH, McDonald JW, Johnston MV. Sensitivity to AMPA induced brain injury transiently peaks during postnatal development. Soc Neurosi Abstr 1990;16:1123
17. Coyle JT, Enna SJ. Neurochemical aspects of the ontogenesis of GABAergic neurons in the rat brain. Brain Res 1976; 111:119–133
18. Palacos JM, Niehoff DL, Kuhar MJ. Ontogeny of GABA and benzodiazepine receptors: effects of Triton X-100, bromide and muscimol. Brain Res 1979;179:390–395
19. Swann JW, Brady RJ, Martin DL. Postnatal development of GABA-mediated synaptic inhibition in rat hippocampus. Neuroscience 1989;28:551–561
20. Haglund MM, Schwartzkroin PA. Seizure-like spreading depression in the immature rabbit hippocampous in vitro. Dev Brain Res 1984;14:51–59
21. Hablitz JJ. Spontaneous ictal-like discharges and sustained potential shifts in the rat neocortex. J Neurophysiol 1987;58: 1052–1065
22. Luhmann HJ, Prince DA. Transient expression of polysynaptic NMDA receptor-mediated activity during neocortical development. Neurosci Lett 1990;111:109–115
23. Swann JW, Smith KL, Brady RJ. Extracellular K^+ accumulation

during penicillin induced epileptogenesis in the CA3 region of the immature rat hippocampus. Dev Brain Res 1986;30: 243–255
24. Schwartzkroin PA. Development of rabbit hippocampus: physiology. Dev Brain Res 1982;2:469–486
25. Haglund MM, Schwartzkroin PA. Role of NA, K pump potassium regulation and IPSPs in seizures and spreading depression in immature hippocampal slices. J Physiol 1990;63:225–239
26. Hanson AJ. Extracellular potassium concentration in juvenile and adult rat brain cortex during anoxia. Acta Physiol Scand 1977;99:412–420
27. Wasterlain CG, Hattori H, Yang C, et al. Selective vulnerability of neuronal subpopulations during ontogeny reflects discrete molecular events associated with normal brain development. In: Wasterlain CG, Vert P, eds. Neonatal seizures. New York: Raven Press, 1990:69–81
28. Jensen FE, Applegate CD, Chansky M. Perinatal hypoxia has longterm effects on seizure susceptibility in rats. Soc Neurosci Abstr 1990;16:1327
29. Jensen FE, Applegate CD, Holtzman D, Burchfiel J. Differential effects of hypoxia versus anoxia on developing rat EEG activity. Soc Neurosci Abstr 1990;15:1346