Acute and Chronic Effects of Unilateral Cerebral Infarction on the EEG and Behavior of the Rat

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Abstract: Acute and Chronic Effects of Unilateral Cerebral Infarction on the EEG and Behavior of the Rat

Anoxic ischemia with resultant unilateral cerebral infarction was produced in the rat by unilateral ligation of the external and common carotid arteries and exposure of the animal to nitrogen. EEG and behavior were studied for one month after the anoxic insult. Infarcted animals showed prominent circling, contralateral flexion postures, and absent visual and tactile placing. At the end of one month the only persistent clinical abnormality was absent contralateral tactile placing. Acutely the EEG showed loss of normal background activity and high voltage slow waves over the infarcted hemisphere. EEG improvement paralleled clinical improvement. At the end of one month only minor asymmetries were observed in the EEG but sleep spindles remained absent on the infarcted side.

Additional Key Words
sleep spindles anoxia slow waves ischemia

In 1950 Hicks exposed rats to nitrogen until the animals became apneic and repeated this procedure two to 60 times. The author found a narrow margin between asphyxia sufficient to kill the animal and asphyxia that just failed to produce cerebral lesions. Approximately one-half of the surviving animals had necrotic lesions in the cortex, gray matter of the corpus striatum, and substantia nigra. Levine in 1960 used a similar technique of exposing rats to nitrogen or nitrous oxide but, in addition, ligated one common carotid artery. In only one rat did carotid ligation without subsequent asphyxia produce a small ischemic lesion in the corpus striatum. The extent of ischemic lesions was variable but usually involved cerebral cortex, corpus striatum, callosal radiation, hippocampus, thalamus, and midbrain on the side of carotid ligation. The hippocampus was the most vulnerable structure, and gray matter was more severely affected than white matter. Frequently, the septum, corpus callosum, medial part of the neocortex, hypothalamus, parts of the thalamus, and the medial part of the corpus striatum were spared.

Using the same rat preparation as Levine, Allen et al. and Clendenon et al. reported cerebral edema within 50 minutes after anoxia. Edema increased during the next 24 hours. Mitochondrial fragmentation was detected as early as one hour after termination of anoxia. Lysosomal enzyme release was either minimal or occurred only three hours after anoxia and did not appear to be a decisive factor in the pathogenesis of cell injury. Complex alterations in glycogen metabolism were demonstrated on the infarcted side and in the adjacent cortex of the contralateral hemisphere. Perfusion of the cerebral circulation with carbon black demonstrated pallor in the dorsal lateral cortex and striatum of the ischemic hemisphere which was grossly evident at three hours after anoxia. In areas of maximal ischemic injury tortuosity and narrowing of the lumen of small arterioles and endothelial swelling of capillaries were apparent.

To our knowledge no acute or chronic electrophysiological studies of this model of anoxic ischemia have been done. The purpose of the present investigation was to study the EEG during anoxia and sequentially for one month thereafter. Clinical observations were made each time the EEG was recorded.

Methods
Forty Sprague-Dawley rats weighing between 300 and 400 gm were used. The animals were anesthetized with intraperitoneal pentobarbital (5 mg per 100 gm body weight). The animal then was placed in a stereotaxic frame, the skull was exposed, and small gold-plated wood screws were inserted in the skull overlying the anterior, central, and posterior cerebral areas. In some animals stainless steel electrodes insulated except at the tip were inserted through nylon screws in the skull into central cortical areas 1 mm deep. Electrodes were separated by 1 mm. All electrodes were connected with insulated copper wires to a Cannon miniature receptacle which was attached to the skull by stainless steel screws. The whole assembly was embedded in

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dental cold cure acrylic. After recovery from anesthesia the EEG was recorded frequently for ten days during wakefulness and sleep. Following adequate healing the animal was anesthetized again and the right common carotid artery ligated. In later experiments, the common and external carotid arteries were separately ligated to reduce collateral flow from the other side. Following ligation, the animals were allowed to recover for approximately one hour. They were then placed in an airtight plexiglass chamber and the electrodes connected via a mating plug to a Grass Model 3B electroencephalograph. Nitrogen was introduced into the chamber at a rate of 2 liters per minute. Usually the rat passed through an excited stage with hyperpnea into coma with respirations below 40 per minute. The rat was kept in this state for as long as possible (10 to 15 minutes) and resuscitated when apnea occurred or when the EEG became flat over both hemispheres. As soon as the animal started to breathe again at a normal rate, nitrogen was again introduced. This procedure was repeated several times and was terminated when resuscitation did not result in reappearance of cerebral electrical activity over the hemisphere on the ligated side. Usually four to six nitrogen exposures were necessary. With each successive nitrogen administration the period during which the animal could be kept comatose and breathing at a rate of 30 to 40 per minute became progressively shorter. Mortality was high and 23 animals could not be resuscitated. Surviving animals were kept alive for at least one month. EEGs during alertness and sleep were recorded almost daily. Three animals died during the first 36 hours after anoxia. Only one animal developed seizures and died in status epilepticus.

EEGs were analyzed visually. At the end of one month the 14 surviving animals were killed and cerebral infarction on the side of carotid ligation verified histologically.

**Results**

During wakefulness the EEG showed predominantly generalized 20 to 50 Hz waves with an amplitude of 10 to 100 microvolts and intermittent 4 to 8 Hz waves with an amplitude of 40 to 150 microvolts. The 4 to 8 Hz activity was clearly not related to respiratory movements or quivering of the jaw. During drowsiness fast activity decreased, and after variable periods of time high voltage (up to 500 microvolts) 6 to 9 Hz spindle-like activity of one to four seconds' duration appeared over all cortical areas, at times more prominently over anterior areas. As sleep progressed, the EEG changed to predominantly high voltage 3 to 6 Hz waves. During all sleep stages the animals could be readily alerted with reappearance of the characteristic waking EEG pattern.

Following carotid ligation and with the animal still minimally anesthetized, the rat was placed in a plexiglass chamber and nitrogen introduced. Attenua-
tion and ultimately loss of 20 to 50 Hz activity were the earliest changes over the ligated hemisphere. After attenuation of fast activity occasionally bursts of high voltage rhythmic 15 to 20 Hz waves appeared followed by irregular polymorphic medium voltage 1 to 3 Hz waves. As nitrogen exposure continued, fast activity attenuated over the nonligated hemisphere and high voltage polymorphic slow waves with a frequency of 1 to 3 Hz appeared (fig. 1). Respiration at this point usually slowed to below 40 per minute. Resuscitation was instituted immediately when the animal either became apneic or all EEG activity disappeared. Fast activity reappeared within a few seconds over the nonligated hemisphere but remained attenuated over the ligated hemisphere. With the rat still comatose but breathing spontaneously at a rate above 50 per minute, nitrogen was reintroduced. The rat was allowed to recover after the fourth nitrogen administration. If at the end of one hour after anoxia EEG activity remained absent or severely attenuated over the ligated hemisphere, the rat was allowed to recover completely. When EEG and clinical recovery had taken place after one hour the whole procedure was repeated for as long as it was necessary. Absence or severe attenuation of EEG activity on the ligated side was accompanied clinically by a tendency to fall toward the contralateral side, prominent forced circling, absence of contralateral visual and tactile placing, and abnormal contralateral flexion postures of the extremities.

Surviving animals displayed persistent circling, abnormal postures and absent visual and tactile placing 24 hours after infarction. At this time the EEG was characterized by persistent attenuation of fast activity over the ligated side, prominent high voltage polymorphic and arrhythmic 1 to 3 Hz waves and infrequent 100 to 200 microvolt spike discharges, rarely seen beyond 48 hours (fig. 2). Sleep spindles were absent on the infarcted side as illustrated in figure 3.

Between 10 and 15 days after infarction most animals appeared quite normal clinically but continued to show a slight tendency to circle toward the infarcted hemisphere. Only an occasional animal showed persistence of abnormal postures. Although visual placing had recovered by this time, tactile placing remained absent. The EEG recorded at this time during the waking state showed minimal attenuation of fast activity and increased 4 to 7 Hz activity over the infarcted hemisphere. Sleep spindles remained absent on the side of carotid ligation.

EEG during wakefulness 24 hours after anoxia. Right carotid ligated. Surface electrodes: 1, 2, 7, 8; cortical depth electrodes: 3, 4, 5, 6. Calibration: horizontal bar one second; vertical bar 100 microvolts. Note occasional spike discharges on right side.
At the end of one month after anoxia, absence of contralateral tactile placing was the only persistent clinical finding. The wake EEG at this time was that of a normal rat as illustrated in figure 4. Sleep spindles, though, remained strikingly absent on the side of carotid ligation as shown in figure 5. Two animals were observed for two months and even at that time sleep spindles had not returned.

Discussion

EEG activity during the waking state in our animals was similar to that observed by Feldman and Robinson. Initially, we placed a reference electrode over the frontal sinus. This electrode, though, could not be used because it recorded respiratory movements as reported by Klingberg et al. Sleep EEG patterns, as observed in the present study, agree with observations by Klingberg and Pickenhain, and Roldan and Weiss. Sleep spindles were distinctly different from movement artifacts secondary to jaw quivering in the awake, relaxed rat.

Nitrogen does not produce narcosis when administered with less than three atmospheres of pressure. Therefore, the behavioral and EEG changes in the present experiments cannot be attributed to nitrogen but have to be secondary to hypoxia. At this time we have insufficient information as to the precise mechanisms leading to infarction which probably are a combination of hypoxia, ischemia and possibly hypotension. The yield and the predictability of cerebral infarction increased after we changed our method from simply ligating the common carotid artery to ligating the common and the external carotid arteries, which must significantly reduce collateral blood flow through the external carotid artery. Common carotid artery ligation alone did not produce EEG changes nor any clinical manifestations of impaired cerebral function in the distribution of the ligated artery. This was true not only acutely but also chronically in animals who were not made anoxic but were observed for several weeks after carotid ligation. This is in contradistinction to Wexler and Sarofim findings that arteriosclerotic and nonarteriosclerotic rats manifested progressively increasing cerebral edema after unilateral common carotid artery ligation alone. Many of their animals died shortly after ligation. When both the common and external carotid arteries were ligated in our study, about 20% of the animals developed ipsilateral attenuation of EEG activity which disappeared within a few days. This
suggests that significant collateral blood flow through the external carotid artery and other channels does occur.

It is difficult to estimate precisely the duration of anoxia required to produce cerebral infarction because repeated nitrogen administrations interrupted by resuscitations were needed. It appears, though, that a total duration of at least 20 minutes of nitrogen administration with the animal comatose and breathing at a rate below 40 per minute is required. Brown and Brierly found heart rates ranging from 420 to 540 per minute in nonanesthetized animals. During anoxia total duration of bradycardia (below 200 per minute) was roughly proportional to the severity of brain damage. In the present study heart rates below 180 per minute which persisted for longer than one minute were usually incompatible with survival of the animal.

Return of EEG activity over the ligated hemisphere less than one hour after termination of the last nitrogen administration was always associated with rapid full recovery. Persistent EEG and clinical abnormalities beyond one hour after anoxia were always associated with cerebral infarction. Histologically, lesions were quite variable, but were most frequent in the neocortex, striatum, and hippocampus, with sparing or less severe involvement of medial cortical portions as reported by Levine and Clendenon et al. All animals which survived beyond 48 hours ultimately improved clinically to the point where the only persistent abnormality at the end of one month was absent tactile placing in the extremities contralateral to the infarct.

During periods of anoxia we did not observe exactly the same sequence of EEG changes as described by Creutzfeldt et al. This probably can be explained by the gradual induction of anoxia in our experiments. We did observe the so-called “free interval” which was of variable duration. The “activation period” did not occur at times and, if it did occur, was quite variable in duration. Consistently, though, we found a period of delta activity which was followed by electrocerebral silence. Hossmann and Sato reported that in ischemia a period of activation followed by electrocerebral silence. During the immediate postanoxic period and during the next few days while the animals continued to show
circling and abnormal posturing the EEG was characterized by delta activity over the infarcted hemisphere, the magnitude of which was directly related to the extent of the infarct. Forty-eight hours postinfarction, delta activity began to decrease and ultimately disappeared. After disappearance of delta activity the EEG on the infarcted side was characterized by excessive 3 to 6 Hz waves. Ultimately the waking EEG returned to normal. The return of normal EEG activity during wakefulness after three to four weeks postanoxia is not surprising, since the extent of infarcts was variable and usually spared the most anterior, most posterior and mesial cerebral structures. The EEG findings are in agreement with similar sequential EEG changes observed in humans with cortical cerebral infarcts.16-18

The rather striking and highly persistent absence of sleep spindles seen as long as two months after anoxia is probably the result of infarction of parts of the thalamus and/or thalamocortical connections. Similar absence of sleep spindles has been shown in humans with deep subcortical destructive processes involving the thalamus unilaterally.19 Cerebral structures giving rise to sleep spindles must be more vulnerable to anoxia than other structures because, regardless of extent of infarction, sleep spindles remained absent after anoxia in all our animals. Further detailed histological studies are needed to delineate these structures.

References
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