Acta Neuropathol (1991) 81: 578 – 587

Acta Neuropathologica

Neuropathology of remote hypoxic-ischemic damage in the immature rat*

J. Towfighi[1], J. Y. Yager[2], C. Housman[1], and R. C. Vannucci[2]

Departments of [1]Pathology (Neuropathology), and [2]Pediatrics (Pediatric Neurology), The Pennsylvania State University College of Medicine, The Milton S. Hershey Medical Center, P.O. Box 850, Hershey, PA 17033, USA

Received September 10, 1990/Accepted December 3, 1990

Summary. This study was undertaken to determine: (a) the duration of hypoxia required to produce brain damage in immature rats with unilateral carotid artery ligation (Levine technique); (b) the regions of immature brain most vulnerable to hypoxia-ischemia (HI); and (c) the neuropathology of the remote HI insult. To this end, 7-day postnatal rats, subjected to unilateral carotid artery ligation combined with hypoxia of varying durations (45, 60, 75 or 90 min), were killed at 30 days of postnatal age and their brains examined by light microscopy. The results indicated that a longer duration of HI was more likely to produce brain lesions and that the extent and severity of the lesions closely correlated with the length of HI. Shorter intervals of HI primarily damaged the cerebral cortex and hippocampus, while longer periods resulted in more extensive damage and were often associated with cavitary lesions of the cerebral hemisphere. Comparison of HI brain damage produced by the Levine technique in immature and adult rats suggested that in immature rats: (a) the cavitary lesions were common; (b) the non-cavitary cortical lesions had a tendency to show a vertical band-like distribution – a pattern never seen in adults; and (c) the lesions often showed mineralization. The similarities between these experimentally produced HI cerebral lesions and those observed in the developing human brain, such as ulegyria and porencephaly, are discussed.

Key words: Brain – Calcification – Hypoxia-Ischemia – Immaturity – Pathology

Cerebral hypoxia-ischemia (HI) remains a major contributor to perinatal mortality and long-term neurological morbidity in survivors [26, 29]. Accordingly, animal models of perinatal HI brain damage have been developed to gain a better understanding of those underlying vascular and metabolic alterations which culminate in tissue injury. One such model is a modification of the Levine preparation [15], whereby 7-day postnatal rats are subjected to a combination of unilateral common carotid artery ligation and the hypoxia produced by the inhalation of 8% oxygen [22]. Exposures to HI of 2- to 3.5-h duration lead to brain damage predominantly in the cerebral hemisphere ipsilateral to the arterial occlusion in up to 92% of animals, with infarction in the majority [17, 21, 22, 30]. The primary goal of the present investigation was to ascertain the minimal duration of HI required to produce tissue injury, and to characterize the nature and distribution of the threshold lesions which arise from HI in the immature rat.

Material and methods

Experimental design

Dated, pregnant Wistar rats were purchased from a commerical breeder (Charles River, Wilmington, MA) and housed in individual cages. Offspring, deliverd vaginally, were reared with their dams until time of initial experimentation at 7 days of postnatal age.

Cerebral HI was induced in 7-day postnatal rats by a previously described technique [22]. Specifically, individual rat pups were lightly anesthetized with halothane (4% induction, 1.0%–1.5% maintenance) during which the right common carotid artery was permanently ligated with 4–0 surgical silk. Upon recovery from anesthesia (10–12 min), the animals were returned to their dams for 3–4 h. Thereafter, groups of three animals were placed in 500 ml air-tight jars partially submerged in a 37 °C waterbath. A gas mixture of 8% oxyen – 92% nitrogen was delivered via inlet and outlet portals. The rat pups were exposed to this gas mixture for 45, 60, 75 or 90 min, following which they were maintained an

* Supported by grant No. #HD 19913 from the National Institute of Child Health and Human Development. Dr. Yager is the recipient of a Fellowship from the R. Samuel McGlaughlin Foundation in Canada

Offprint request to: J. Towfighi (address see above)

additional 15 min in open jars in the waterbath. Thereafter, the rat pups were returned to their dams and reared until 30 days of postnatal age, at which time they were anesthetized with pentobarbital (100 mg/kg body weight) and immediately killed by decapitation. Since brain damage does not occur in rat pups exposed to either carotid artery ligation or hypoxia alone [22], control littermates underwent neither arterial ligation nor hypoxia.

Neuropathological methods

The brains were removed fresh from their skulls and immediately submerged in a 1:1:8 mixture of formaldehyde: acetic acid: methanol (FAM). After 48 h of fixation in FAM, the brains were examined grossly for external abnormalities. Thereafter, the FAM-fixed brains were cut coronally and two blocks, each approximately 2 mm thick, were selected for paraffin embedding. One block contained the median eminence and the other, taken just anterior to this, contained the anterior commissure. Subserial sections of 6 μm were stained with hematoxylin and eosin (H & E), Luxol fast blue – periodic acid-Schiff (LFB-PAS), and glial fibrillary acidic (GFA) protein by an immunoperoxidase technique. Prussian blue reaction and Perls stain for iron and von Kossa's calcium stain were used in specimens with HI damage.

The extent and distribution of brain damage were determined in a blind fashion by microscopic examination of all subserial sections. The type and distribution of lesions in two sections taken at specific levels from each brain were recorded and scored (see Results). One section was taken from midway between the level of the body of the anterior commissure and the level of the genu of the corpus callosum (anterior level), and the other from the level of the median eminence (posterior level); the anterior level included primarily the cerebral cortex and striatum; the posterior level included the cerebral cortex, hippocampus, amygdaloid nucleus, putamen, globus pallidus and thalamus.

Morphometric study was carried out on symmetric coronal sections taken at the posterior level. The entire section was projected onto a screen connected to a planimeter (Nikon Microplan II). The total sectional area of each cerebral hemisphere was measured and the ratio of the cerebral hemisphere contralateral (left) and ipsilateral (right) to the arterial occlusion was calculated for each brain. Included in the measurements was only the brain tissue with or without gliosis; the ventricles, choroid plexi and cystic areas in brain with infarcts were excluded.

Results

A total of 41 animals from 7 litters (7–11 animals/litter) were exposed to cerebral HI for 45, 60, 75 or 90 min. All animals survived the exposure and were returned to their dams. Three rat pups (1 each at 60, 75 and 90 min) sustained a delayed death, leaving 8 animals in the 45-min group and 10 animals in each remaining group for neuropathological analysis. No clinical abnormalities were noted in the surviving animals except for mild ptosis of the eyelid on the side of the carotid artery ligation in a few animals. This abnormality was most likely due to damage to the pericarotid sympathetic nerve fibers incurred at the time of the ligation of the carotid artery.

Neuropathology

Gross examination. Gross pathological alterations ranged from no damage to infarction with loss of tissue, predominantly in the distribution of the middle cerebral artery (MCA). Mild to moderate atrophy of the cerebral hemisphere ipsilateral to the common carotid artery ligation was apparent in two of ten brains from animals exposed to 60 min of HI and in three of ten brains from animals exposed to 75 min of HI. Of the ten animals subjected to 90 min of HI, one brain exhibited mild atrophy and four brains exhibited cystic infarcts. The remaining brains including all those brains from animals exposed to 45 min of HI were considered normal. One brain assessed as normal by gross inspection (brain A from a 60-min HI animal) showed mild histological abnormalities, while a single brain thought to be mildly atrophic on gross inspection (brain from a 75-min animal) was normal on histological examination.

Histology. Brains from rats that were exposed to 45 min of HI showed no abnormalities. Histological abnormalities were present in three of ten brains from rats with 60 min of HI, in two of ten brains from rats with 75 min of HI, and in five of ten brains from rats with 90 min of HI (Table 1). In general, the lesions were less extensive in the 60- and 75-min animals compared to the 90-min animals. Moreover, larger infarcts resulting in cavitation (cystic infarct) were limited to the 90-min specimens (four of five brains with lesions). The incidence of damage to specific structures within the ipsilateral cerebral hemisphere in decreasing order of frequency were: cortex = hippocampus > striatum ≥ globus pallidus ≥ thalamus ≥ amygdaloid nucleus ≥ white matter. The type and distribution of lesions in different brain regions of the ten damaged rats are discussed briefly.

Cerebral cortex. In posterior levels, the cortex was damaged in all brains and lesions ranged from small areas of neuronal necrosis, neuronal loss and astrogliosis to large cystic infarcts. In anterior levels, all damaged brains except one exhibited pathological alterations. Non-cavitary lesions on coronal sections usually appeared as multiple thin strips or bands oriented perpendicular to the pial surface with the narrow tip located in the outer layers (layer 2 or 3) and the wide base located in the deeper layers (5 and 6). Depending upon the severity of the lesions, the bands converged and joined at the base to produce a continuous layer of

Table 1. Extent of brain damage in rats subjected to hypoxia-ischemia at 7 days of age

Duration hypoxia-ischemia	Rat	Cortex		Hippocampus					Amygd nucleus	Striatum		Globus pallidus	Thalamus	Histological score[a]
		ant	post	CA1	CA2	CA3	CA4	FD		ant	post			
60 min														
	A	+	+	−	+	+	−	−	−	−	−	−	−	4
	B	+	+	−	+	+	−	−	−	+	−	+	−	6
	C	+	+	+	+	+	−	−	+	+	+	+	+	10
75 min														
	D	+	+	+	+	+	−	−	−	+	−	−	−	6
	E	+	+	+	+	+	+	−	+	+	+	+	+	11
90 min														
	F	−	+	+	+	+	+	−	−	−	+	+	+	8
	G	+	+[b]	+	+	+	+	+	+	+	+[b]	+[b]	+	15
	H	+[b]	+[b]	+	+	+	+	+	+	+	+[b]	+[b]	+	16
	I	+[b]	+[b]	+	+	+	+	+	+[b]	+[b]	+[b]	+[b]	+	18
	J	+[b]	+[b]	+	+	+	+	+	+[b]	+[b]	+[b]	+[b]	+[b]	19

[a] Estimate of the severity of damage (see Text)
[b] Cystic infarct
Ant = Anterior; Post = posterior; FD = fascia dentata

laminar necrosis (Figs. 1, 2). These rows of damaged vertical bands interdigitated with the non-damaged overlying cortex. The latter on serial sections appeared to consist of short, finger-like projections that originated in the cortical layer 2 or 3 and dipped to some distance into the underlying damaged cortex. This tridimensional concept was confirmed on sections close to the anterior or posterior poles of the cerebral hemisphere, where some of these finger-like projections of non-damaged cortex were cut perpendicular to their long axis and appeared as circular profiles separated by damaged cortex (Fig. 3). The latter often contained one blood vessel in the center. The damage in brains with non-cavitary lesions was limited to the MCA distribution in the lateral aspect of the hemispheres, and in three animals extended into the adjacent pyriform cortex. There was variable reduction in the thickness of the cortex with compensatory dilation of the lateral ventricle that depended in severity upon the extent of the neuronal loss and gliosis. In addition to neuronal loss and gliosis, there were often neuronal ghosts present in

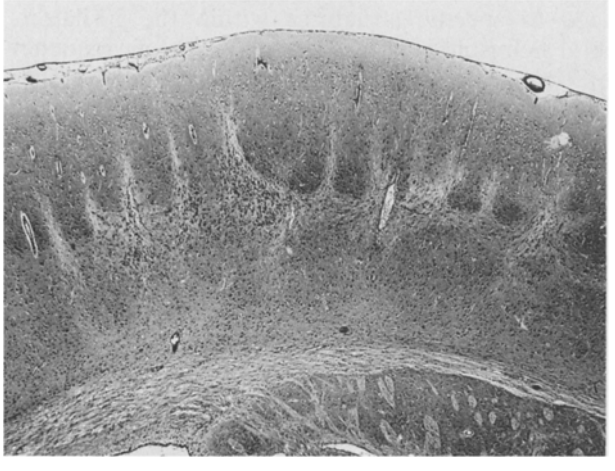

Fig. 1. Coronal section, ipsilateral cerebral cortex of a 30-day postnatal rat following unilateral carotid artery ligation and 75 min of hypoxia at 7 days of age. Areas of vertical band-like necrosis join at the deeper layers to form a continuous layer. Hematoxylin and eosin stain, × 25

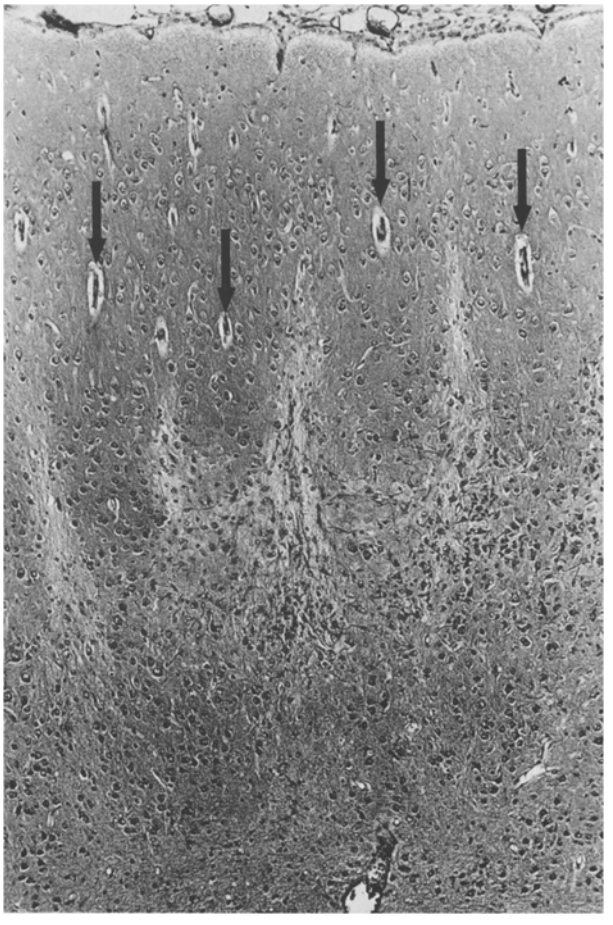

Fig. 2. Same as Fig. 1. Finger-like projections (columns) of preserved cortex extend into the deeper necrotic region. Note the presence of an arteriole (*arrows*) in the core of each preserved column. Hematoxylin and eosin stain, × 75

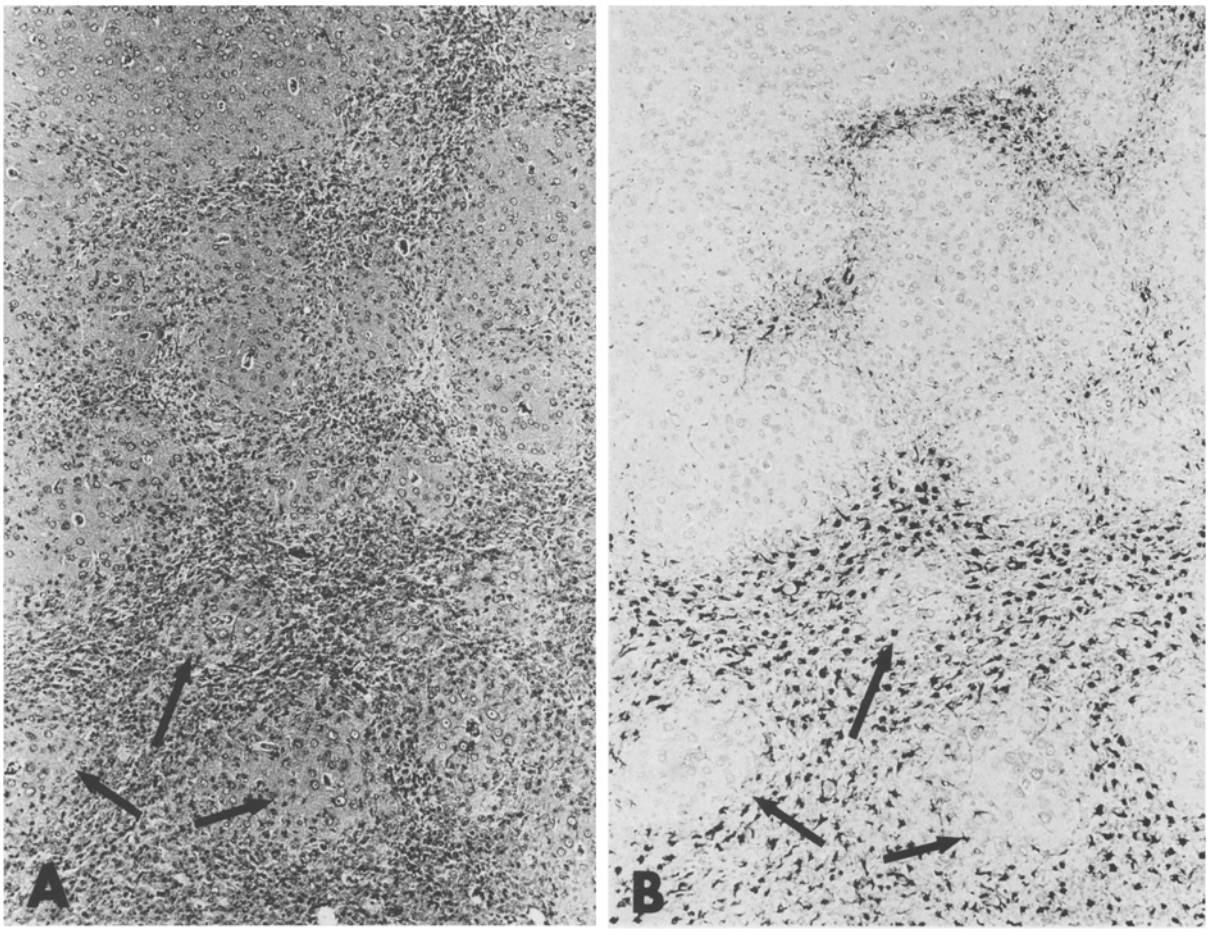

Fig. 3A, B. Ipsilateral cerebral cortex with hypoxic-ischemic damage. Cross sections of tip of finger-like projections of preserved cortex into deeper layers are seen as circular profiles (*arrows*) separated by damaged areas of astrocytosis. **A** Hematoxylin and eosin, **B** immunoperoxidase for glial fibrillary acidic (GFA) protein; **A, B** × 85

the regions of laminar necrosis and at the bases of necrotic vertical bands.

In brains with cystic lesions, the damaged areas were larger and not only involved the entire thickness of the cortex and white matter but also extended to the territories of the anterior and posterior cerebral arteries with involvement of the pyriform cortex. Each cavity was bound laterally by an extremely thin, transparent membrane consisting of pia and a narrow zone of gliosis. Deeply, the thin gliotic wall of the cavity communicated with the lumen of a dilated lateral ventricle via a large or small opening (Figs. 4, 5). The presence of ependymal lining (single layer of flat to cuboidal cells) extending into and partly lining the cavity of the infarcted area (Fig. 4B) indicated that the communication between the cavity of the cystic infarct and the lateral ventricle was real rather than representing an artifact secondary to brain removal or sectioning. The cavity was separated from the adjacent normal cortex by a transitional zone showing varying degrees of neuronal damage described above (see Figs. 4A, 5). Mineralization was often present in the damaged cortical regions especially in the areas bordering the cystic infarcts. The deposits were mild and could not be seen in H & E or Prussian blue stains, but could be demonstrated by PAS, Perls stain for iron or von Kossa stain for calcium as diffuse fine granular deposits.

Hippocampus. This structure was involved in all damaged animals. The CA2 and CA3 regions in their lateral aspects were most consistently damaged. Indeed, in two animals these sectors were the only two regions of hippocampal damage (Fig. 6). The CA1 region also was damaged in the majority of the animals. In mildly damaged areas of CA1, neuronal necrosis and astrocytosis preferentially involved the outermost neurons (Fig. 7). The CA4 region, medial aspect of CA3, and fascia dentata were relatively spared in animals with non-cavitary cortical lesions, whereas these hippocampal regions were often involved in brains that had cavitary lesions of the cortex, in which specimens of the hippocampi were shrunken and gliotic (Figs. 5, 8). Mineralization was present in the damaged areas in most of the brains. These deposits were often dense and, unlike those in cerebral cortex, could be seen in H & E stain.

White matter. The white matter was infarcted only in those brains that exhibited cystic cortical infarcts. In damaged brains without infarcts, the subcortical white

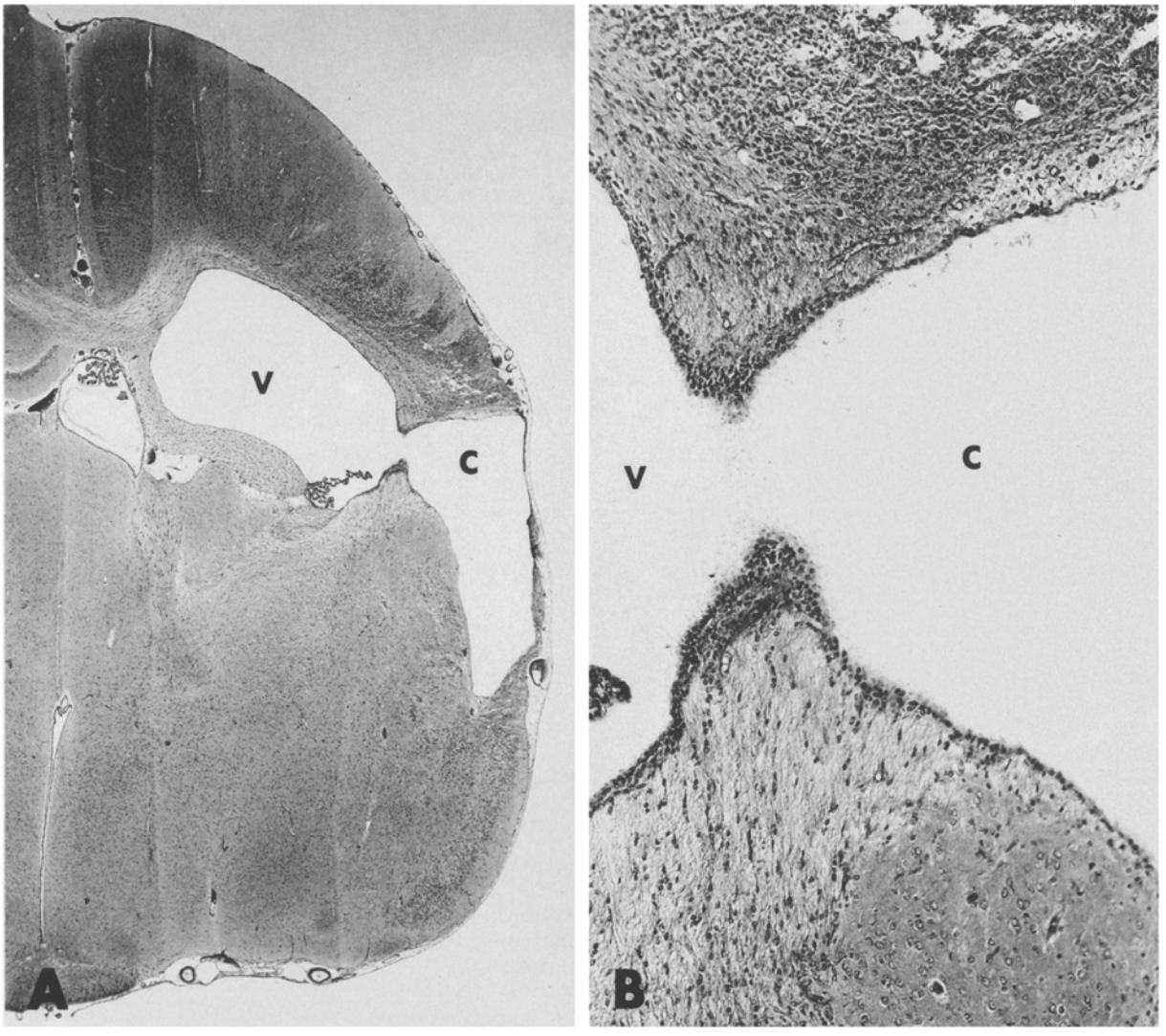

Fig. 4A, B. Coronal section of the ipsilateral cerebral hemisphere of a 30-day postnatal rat following 90 min of hypoxia-ischemia at 7 days of age. **A** Cavitary lesion (*c*) communicates with a dilated lateral ventricle (*v*). Cortex bordering superior aspect of cavity shows transition from confluent to band-like necrosis. **B** Junction between the cavity (*c*) and ventricle (*v*) in higher magnification shows ependymal lining extending into the cavity. **A, B** Hematoxylin and eosin; **A** × 16; **B** × 100

matter and corpus callosum showed a mild pale staining for myelin and a mild astrocytosis bilaterally. The latter was often slightly accentuated on the side of the carotid artery ligation.

Striatum. This region was involved in eight of ten damaged brains. Generally, the involvement was more severe in the anterior level than the posterior level, and the lateral aspect was more often damaged than the medial aspect (Fig. 9). In brains with milder damage, the involved areas were shrunken and gliotic. Mild degrees of mineralization were present only in the posterior level sections and was seen in three brains. In brains with more severe damage, the striatum was partly or totally replaced with a cavity that had become incorporated into a larger one resulting from infarction of other structures within the hemisphere.

Globus pallidus. This was involved in eight of ten damaged brains and the lesions were similar to but generally milder than those described for the striatum.

Thalmus. This structure was involved in seven of ten damaged brains. Generally, the lateral and ventral groups of nuclei were most severely injured. Damaged areas were shrunken and gliotic and always showed moderate to dense mineralization (Figs. 5, 8). No cavitary lesions were seen except for one brain that had a microscopic infarct.

Amygdaloid nucleus. This was involved in six of ten damaged brains. Central nuclei appeared to be most severely involved. Mineralization was mild and present focally in two brains.

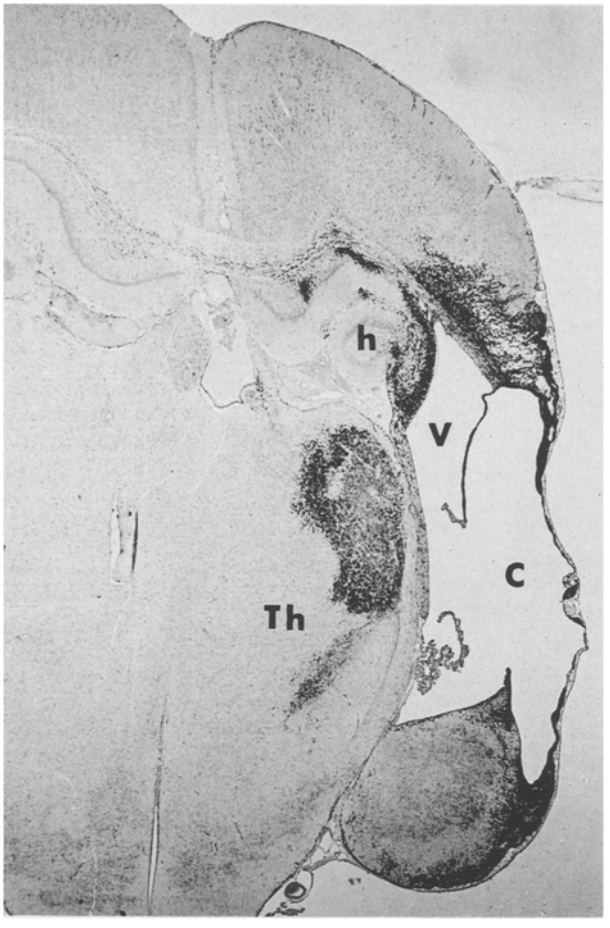

Fig. 5. Ipsilateral cerebral hemisphere of a rat with 90 min of hypoxia-ischemia showing a cavitary lesion (*c*) communicating with the dilated lateral ventricle (*v*) surrounded by astrocytosis. Hippocampus (h) is shrunken and gliotic. Thalamus (*Th*) shows areas of dense astrocytosis in its dorsolateral aspect. Immunoperoxidase for GFA protein, × 15

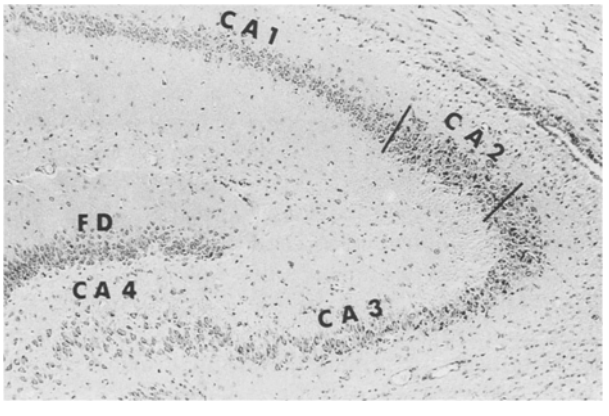

Fig. 6. Ipsilateral hippocampus of a rat with 60 min of hypoxia-ischemia showing minimal damage localized to the lateral aspects of CA3 and CA2 regions. Immunoperoxidase for GFA protein × 60

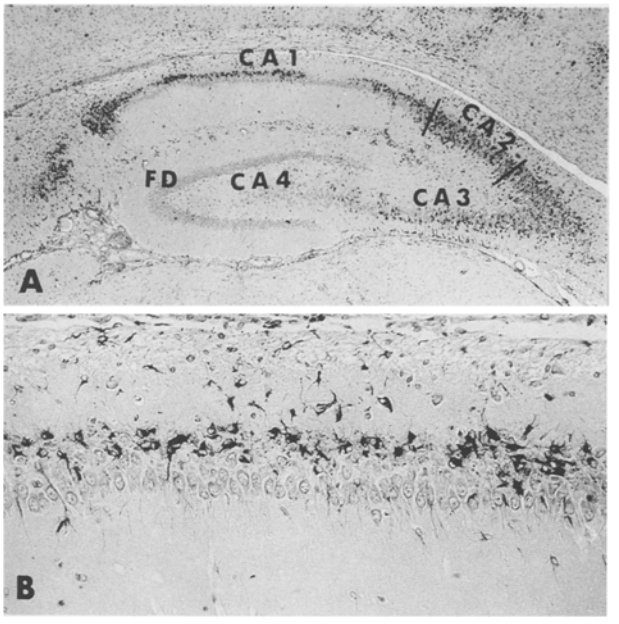

Fig. 7A, B. Coronal section, ipsilateral dorsal hippocampus of a 30-day postnatal rat following 75 min of hypoxia-ischemia at 7 days of age. **A** Severe neuronal loss and astrocytosis of CA2 with relative preservation of fascia dentata (*FD*), CA4, medial CA3 and parts of CA1 sectors. **B** Higher magnification of CA1 region with partial involvement showing neuronal loss and astrocytosis in the outer aspect. **A, B** Immunoperoxidase for GFA protein; **A** × 23; **B** × 145

Table 2. Severity of hypoxic-ischemic brain damage determined by morphometry and by histological scoring

Duration of hypoxia-ischemia[a]	L/R hemispheric ratio mean ± SD (*n*)	Histological score[b]
45 min:		
non-damaged	0.997 ± 0.024 (8)	0
60 min:		
non-damaged	1.024 ± 0.037 (7)	0
damaged A	1.143	4
B	1.156	6
C	1.140	10
75 min:		
non-damaged	1.015 ± 0.027 (8)	0
damaged D	1.348	6
E	1.284	11
90 min:		
non-damaged	1.016 ± 0.032 (5)	0
damaged F	1.261	8
G	2.406	15
H	2.581	16
I	2.602	18
J	4.244	19

[a] Letters of identification for individual rats (A to J) with histological evidence of brain damage

[b] Higher numbers indicate more extensive damage (see Table 1 and text)

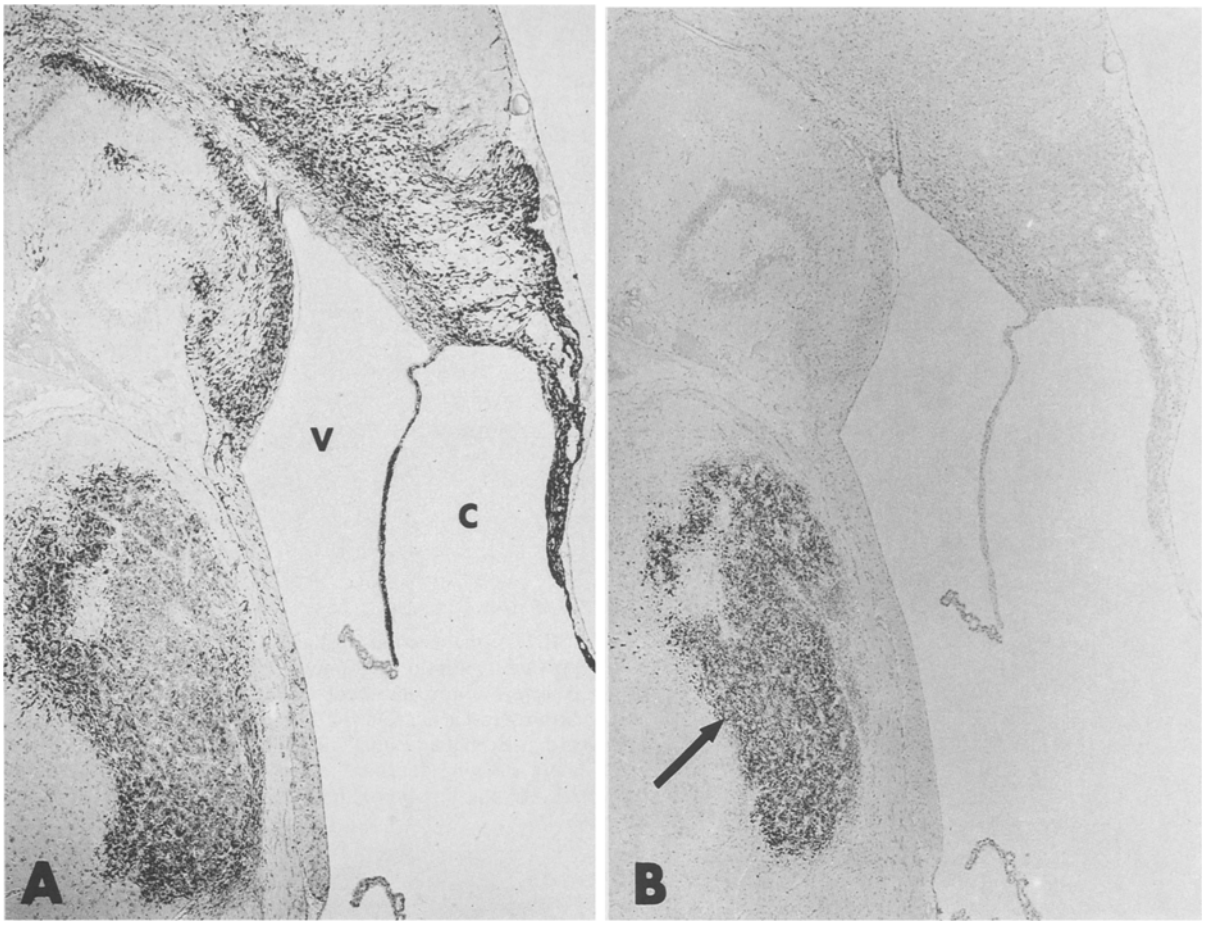

Fig. 8. **A** Same as Fig. 5 showing details of the damaged structures surrounding the cavity (*c*) and lateral ventricle (*v*). **B** Stain for calcium in a section from the same region as **A** showing dense deposits in the dorsolateral thalamus (*arrow*). **A** Immunoperoxidase for GFA protein; **B** von Kossa stain; **A, B** × 35

Morphometric studies. The degree of loss of cerebral hemispheric area on coronal section as calculated by the ratio of the non-damaged (contralateral) hemisphere to the damaged (ipsilateral) hemisphere at the posterior level roughly correlated with the duration of HI except in one animal of the 90-min group which showed a mild lesion (animal F in Table 2). To validate this observation, the results were compared to those obtained by histological scoring. The histological scoring in each brain was obtained by giving a score of 1 to each involved region (Table 1). An additional score of 1 was given to those regions exhibiting cystic infarction. Correlation of coefficient analysis revealed close correspondence between the severity of brain damage determined histologically and by mophometry ($r = 0.87$; $P < 0.001$).

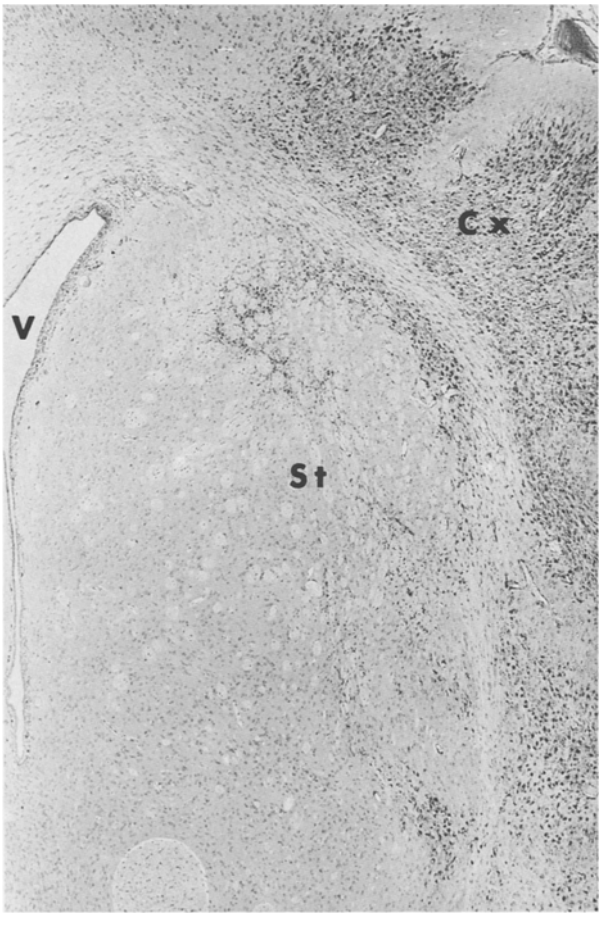

Fig. 9. Ipsilateral striatum (*St*) of a rat with 90 min of hypoxia-ischemia showing patchy neuronal damage and astrocytosis involving mainly the lateral aspect. Overlying cortex (*Cx*) is also involved. *v:* lateral ventricle. Immunoperoxidase for GFA protein, × 34

Discussion

The major objectives of the present investigation were twofold: (1) to determine the duration of HI required to produce tissue injury in a well-established model of perinatal HI brain damage; and (2) to ascertain those regions of immature brain which are most vulnerable to damage. Not unexpectedly, the data presented here show that the longer the interval of HI exposure, the greater the likelihood that brain damage will occur with increasing severity thereafter (Table 1). No cerebral lesions were present in rats exposed to 45 min of HI; whereas 25 % (5/20) developed non-cystic lesions with 60–75 min of exposure; and 50 % (5/10) with 90 min of HI had lesions that were more extensive and often cavitary (4/5). Longer intervals (2–3.5 h) of HI are associated with brain damage in up to 92 % of animals, with widespread infarction in the majority [17, 21, 22, 30].

Our histological scoring and morphometric analyses of the damaged and undamaged brains showed an excellent correlation between the severity of the histopathological alterations and the extent of cerebral atrophy and tissue loss (Table 2). Those brains without histological evidence of damage showed no correlation between the duration of HI and the cerebral interhemispheric ratio. Moreover, there was a sharp demarcation between the interhemispheric ratios of the histologically normal brains and those of the brains with the mildest damage. Accordingly, HI insufficient to produce histologically confirmed brain damage also has no deleterious effect on brain growth per se.

The pattern of distribution of the lesions in brains of immature rats was similar to that seen in adults [6, 15] but with notable exceptions. In immature rat brain, the non-cavitary lesions seen in cerebral cortex typically consisted of a combination of vertical bands and laminar patterns. Subcortical and periventricular white matter failed to show damage except in those brains with severe lesions extending from cerebral cortex to white matter. Prominent white matter injury has been observed in immature rat brain, when HI exposures are extended to 3.0–3.5 h and survivals shortened to 15–50 h [22]. All zones of hippocampus were sensitive to hypoxia-ischemia except for the CA4 (hilar) region, fascia dentata and medial region of CA3. The lateral CA2 and CA3 zones were consistently involved, and in two animals these sectors were the only damaged areas of hippocampus. Interestingly, the outer aspect of CA1 was more severely involved than the innermost region.

A striking feature of HI damage within the ipsilateral cerebral cortex was the presence of cortical bands of damaged neurons oriented at right angles to the pial surface. These narrow bands, in severely damaged regions of cortex, became widened and joined at their bases in the deeper cortical layers to enclose short finger-like projections of the normal overlying cortex. This pattern of cortical lesion that has been referred to as radial or columnar cortical damage [22] is already apparent following 15–50 h of HI in the immature rat, but its counterpart has never been reported in adult rat brain. In the latter, a comparable HI stress results in damage along specific cortical layers (laminar necrosis) which may show accentuation at arterial border zones [6, 15].

The reason for the observed differences in the pattern of cortical HI damage between immature and adult animals is not clear. It is likely that the vertical band-like pattern seen in the cerebral cortex of the immature animal is related to the known structural immaturity of this region. Specifically, there is a high neuronal packing density with dendrites not yet fully formed [5, 12] and a capillary bed that has not attained its adult density [2, 10]. It is also possible that in the immature cortex, metabolic differences between laminae and individual neurons are minor or even absent. If so, the distribution of HI damage is likely to be determined to a great extent by the pattern of cortical vascular architecture. In this regard, the observed variations in both width and depth of the finger-like projections of non-damaged regions may reflect corresponding variations in the caliber and length of penetrating arteries.

A band-like or nodular arrangement of damaged and preserved neurons in cerebral cortex has been encountered in human autopsied brains variably labelled as ulegyria [13] or nodular cortical sclerosis [9]. The antecedent perinatal events in these cases often were obscure and the lesions not infrequently ascribed to developmental abnormalities of the brain. However, more recent investigations have demonstrated an association between cerebral HI occurring in the immediate newborn period and a columnar arrangement of damage in cerebral cortex [13, 20].

It is noteworthy that subcortical and periventricular white matter was no more vulnerable to HI damage than either cerebral cortex or hippocampus, despite the functional and anatomic immaturity of 7-day postnatal rat brain. White matter necrosis, with relative sparing of gray matter structures, is a prominent feature of HI in experimental and human fetuses and in prematurely born infants [3, 8, 14, 24]. In premature human infants, the lesions are typically periventricular in location; whereas in infants born closer to term, the topography of the necrotic foci shifts to subcortical areas, often with associated selective neuronal necrosis or cavitary infarction of the overlying cerebral cortex, as seen in the immature rat (present study).

The cavitary lesions involving cerebral cortex and underlying white matter had thin gliotic walls and communicated deeply with the lumen of the lateral ventricle. They exhibited a smooth inner surface and were partially lined with ependymal cells. This change is similar to porencephaly in the human brain, the origin of which is thought to be an early *in utero* HI damage. The porencephalic cavity is typically covered by a thin arachnoid membrane; it communicates with the ventricle and has a smooth inner surface [13]. However, it should be noted that porencephaly by its strict definition, unlike the cavitary HI lesions in immature rats, does not have astrocytosis in its wall, and the adjacent cortex instead of columnar necrosis shows developmental abnormalities such as polymicrogyria. This difference

may be related to the stage of brain development at the time of the insult and to the animal species under study. In 7-day postnatal rats which are capable of a glial response, HI will result in a lesion different from that of the early human fetal brain which is incapable of such a response. Indeed, instances of porencephalies with polymicrogyria in the formative stage have been observed as early as 24–26 weeks of gestation [13, 19].

Another observation that has not been addressed in adult models of HI brain damage which was prominent in immature rat brain was the presence of mineralization of the damaged regions, especially in deeper structures such as hippocampus, thalamus, posterior putamen and globus pallidus. Unlike immature rat brain, HI brain lesions in adults, including humans, show little tendency for mineralization [4, 6, 15]. However, mineralization (dystrophic calcification) is a frequent accompaniment to perinatal cerebral lesions occurring in human infants [1, 13, 18].

The reason for immature neurons to undergo dystrophic calcification is unknown. Norman [18] has suggested that the reaction may reflect a sublethal injury to the neuron, a chronic form of cell death, or the unavailability of lytic enzymes. Polymorphonuclear leukocytes, which release lytic enzymes, are absent in small anoxic lesions in neonates nor are they seen in immature rat brain undergoing HI brain damage. Furthermore, the brains of fetal monkeys show a scant polymorphonuclear response to various irritants [23]. Other factors, including the therapeutic administration of calcium salts, may also contribute to the formation of cerebral calcifications. Changaris et al. [7] have found a direct correlation between the average daily dosage of parenterally administered calcium gluconate and the presence and extent of cerebral calcification in asphyxiated newborn infants.

Of the numerous metabolic perturbations (increased anerobic consumption of glucose, disruption of high-energy phosphate reserves, intracellular calcium accumulation) which occur during HI in the immature rat [25, 28, 31], regional alterations in the oxidation/reduction (redox) state of the tissue and enhanced calcium flux into brain predict most accurately the topographic pattern of the neuropathological alterations. Welsh et al. [31] examined the redox state of immature rat brain undergoing HI by the technique of reflectance fluorometry. Alterations in regional fluorescence, representing the intra-cellular accumulation of NADH, were prominent in both cerebral cortex and hippocampus, especially the CA1 and CA3 sectors. A banded pattern of NADH fluorescence was apparent in cerebral cortex, which mimics closely the distribution of neuronal necrosis seen in this region (see Results). The close correspondence between altered NADH fluorescence and neuropathological outcome suggests an important role for intracellular acidosis in the pathogenesis of perinatal HI brain damage, albeit not necessarily lactacidosis [27, 31].

Disturbances in intracellular calcium homeostasis in brain also characterize the immature rat subjected to cerebral HI [25]. Indeed, an excessive accumulation of calcium in neuronal tissues may represent the "final common pathway" not only for HI, but for other forms of acute brain damage as well [11, 16]. Stein and Vannucci [25] subjected 7-day postnatal rats to cerebral HI either prior to or following a subcutaneous injection of $^{45}CaCl_2$. During HI, calcium flux into brain was prominent in cerebral cortex, hippocampus, striatum, and thalamus with minimal or no uptake into subcortical or periventricular white matter. During the first 5 h of recovery, $^{45}CaCl_2$ radioactivity in all brain regions was low, but thereafter increased progressively over 72 h. As during HI, the distribution of radioactivity was most prominent in those regions known to be vulnerable to HI injury. Within individual structures, calcium accumulation was most intense in areas corresponding to the brunt of tissue destruction seen pathologically; including a combined laminar and radial distribution of calcium in cerebral cortex and a predilection for the CA1, CA2 and CA3 sectors of the hippocampus and for the lateral aspects of the striatum and thalamus. The findings indicate a close spatial association between calcium accumulation in brain during HI and the ultimate neuropathological outcome and implicate a disruption of intracellular calcium homeostasis as a major factor in the evolution of perinatal HI brain damage. Furthermore, the progressive secondary accumulation of calcium during the recovery period occurs in parallel with tissue destruction and mineralization.

References

1. Ansari MQ, Chincanchan CA, Armstrong DL (1990) Brain calcification in hypoxic-ischemic lesions: an autopsy review. Pediatr Neurol 6: 94–101
2. Bär T, Wolff JR (1976) Development and adult variations of the wall of brain capillaries in the neocortex of rat and cat. In: Cervos-Navarro J, Betz E, Matakas F, Wullenweber R (eds). The cerebral vessel wall. Raven Press, New York, pp 1–6
3. Banker BW, Larroche JC (1962) Periventricular leukomalacia of infancy. Arch Neurol 7: 386–410
4. Brierley JB, Graham DI (1984) Hypoxia and vascular disorders of the central nervous system. In: Adams JH, Corsellis JAN, Duchen LW (eds) Greenfields, neuropathology, 4th edn. Wiley, New York, pp 125–156
5. Brizzee KR, Vogt J, Kharetchko X (1964) Postnatal changes in glia/neuron index with a comparison of methods of cell enumeration in the white rat. Prog Brain Res 4: 136–149
6. Brown AW, Brierley JB (1968) The nature, distribution and earliest stages of anoxic-ischemic nerve cell damage in the rat brain as defined by the optical microscope. Br J Exp Pathol 49: 87–106
7. Changaris DG, Purohit DM, Balentine JD, Levkoff AH, Holden AEC, Dean DL, Biggs PJ (1984) Brain calcification in severely stressed neonates receiving parenteral calcium. J Pediatr 104: 941–946
8. Clapp JF, Peress NS, Wesley M, Mann LI (1988) Brain damage after intermittent partial cord occlusion in the chronically instrumented fetal lamb. Am J Obstet Gynecol 159: 504–509
9. Courville CB (1971) Birth and brain damage. Courville, Pasadena
10. Craigie EH (1925) Postnatal changes in vascularity in the cerebral cortex of the male Albino rat. J Comp Neurol 39: 301–324

11. Deshpande JK, Siesjö BK, Wieloch T (1987) Calcium accumulation and neuronal damage in the rat hippocampus following cerebral ischemia. J Cereb Blood Flow Metab 7: 89–95
12. Eayrs JT, Goodhead B (1959) Postnatal development of the cerebral cortex in the rat. J Anat 93: 385–402
13. Friede RL (1989) Developmental neuropathology, 2nd edn. Springer-Verlag, New York, pp 82–97, 535–545
14. Leech RW, Olson MI, Alvord EC (1979) Neuropathologic features of idiopathic respiratory distress syndrome. Arch Pathol Lab Med 103: 341–343
15. Levine S (1960) Anoxic-ischemic encephalopathy in rats. Am J Pathol 36: 1–17
16. Meldrum BS (1986) Cell damage in epilepsy and the role of calcium in cytotoxicity. Adv Neurol 44: 849–855
17. Mujsce DJ, Towfighi J, Stern D, Vannucci RC (1990) Mannitol therapy in perinatal hypoxic-ischemic brain damage. Stroke (in press)
18. Norman MG (1978) Perinatal brain damage. Perspect Pediatr Pathol 4: 41–92
19. Norman MG (1980) Bilateral encephaloclastic lesions in a 26 week gestation fetus: effect on neuroblast migration. Can J Neural Sci 7: 191–194
20. Norman MG (1981) On the morphogenesis of ulegyria. Acta Neuropathol (Berl) 53: 331–332
21. Palmer C, Vannucci RC, Towfighi J (1990) Reduction of perinatal hypoxic-ischemic brain damage with allopurinol. Pediatr Res 27: 332–336
22. Rice JE, Vannucci RC, Brierley JB (1981) The influence of immaturity on hypoxic-ischemic brain damage in the rat. Ann Neurol 9: 131–141
23. Schwartz LW, Osburn BI (1974) An ontogenic study of the acute inflammatory reaction in the fetal rhesus monkey. Lab Invest 31: 441–453
24. Shuman RM, Selednik LJ (1980) Periventricular leukomalacia: a one-year autopsy study. Arch Neurol 37: 231–235
25. Stein DT, Vannucci RC (1988) Calcium accumulation during the evolution of hypoxic-ischemic brain damage in the immature rat. J Cereb Blood Flow Metab 8: 834–842
26. Vannucci RC (1989) Acute perinatal brain injury: hypoxia-ischemia. In: Cohen WR, Acker DB, Friedman EA (eds) Management of labor. Aspen Publishers, Rockville, pp 183–244
27. Vanucci RC (1990) Experimental biology of cerebral hypoxia-ischemia: relation to perinatal brain damage. Pediatr Res 27: 317–326
28. Vannucci RC, Christensen MA, Stein DT (1989) Regional cerebral glucose utilization in the immature rat: effect on hypoxia-ischemia. Pediatr Res 26: 208–214
29. Volpe JJ (1987) Neurology of the Newborn II. W. B. Saunders, Philadelphia
30. Voorhies TM, Rawlinson D, Vannucci RC (1986) Glucose and perinatal hypoxic-ischemic brain damage in the rat. Neurology 36: 1115–1118
31. Welsh FA, Vannucci RC, Brierley JB (1982) Columnar alterations of NADH fluorescence during hypoxia-ischemia in immature rat brain. J Cereb Blood Flow Metab 2: 221–228