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THE POSTNATAL CHANGES IN THE VASCULARITY IN THE INFERIOR COLLICULUS OF THE BRAINSTEM OF THE YOUNG RAT

A Thesis

Presented to the Faculty of Graduate Studies,
University of Manitoba, in Partial Fulfilment
of the Requirements for the Degree of
Master of Science

by

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October, 1988

ISBN 0-315-48014-9

THE POSTNATAL CHANGES IN THE VASCULARITY IN THE INFERIOR COLLICULUS OF THE BRAINSTEM OF THE YOUNG RAT

BY

DONA LEE ELIZABETH ANDREW

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

ACKNOWLEDGEMENTS

I would like to thank all the members of the academic and the technical staff of the Department of Anatomy for their support and assistance in the preparation of this thesis. I would also like to thank the Department for awarding me the Anatomical Research Fund Graduate Student Scholarship for the years 1984 to 1987. I would especially like to thank and acknowledge the technical expertise and the encouragement given to me by Mrs. S. Pylypas and the photograghic expertise of Mr. R. Simpson.

I would also like to express my thanks to the following people for their assistance in the preparation of this thesis:

Mrs. M. Cheang, Biostatistic Unit
Dr. R. Shiu, Physiology
Mr. D. Dubik, Physiology

I would like to express my thanks and appreciation to my mother for her encouragement and her unfailing belief in me. Finally, a very special thank you to Dr. J. A. Paterson, my advisor, for sharing her knowledge with me and for all of her support and encouragement that she gave so freely to me.

ABSTRACT

The inferior colliculus in the rat midbrain is an auditory relay center whose functional maturation occurs postnatally. Our main objective in studying the central region of the normal developing rat inferior colliculus by morphometry was to examine changes in vascularity and in nuclear profile density and to compare this region with another gray matter brain region. The inferior colliculus from aldehyde-perfused Sprague-Dawley rats (at 5 days, 9 days, 14 days and 24 days postnatally) was analysed by light microscopy of semi-thin plastic sections. The central region (containing mostly central nucleus) was sampled at five levels representing its entire rostrocaudal extent. Layers IV and V of the left frontal cortex were sampled in the 5 and 24 day old groups. Patent blood vessel profiles were counted and classified according to their internal diameter in cross-section and to their profile orientation. Counts of nuclear profiles were also made. The small ($\leq$ 10 microns) cross-sectional vessel profiles increased over five-fold in number per unit area from 5d to 24d postnatally in the inferior colliculus. Correspondingly, the vascular volume density (estimated by differential point counting) increased. There were minimal differences found in the vascularity between the cortex and the inferior colliculus in the 5 day old group. There were significant differences between the two regions in vascularity by 24

days old with the cortex having fewer small vessel profiles per unit area. There was a decrease in the number of neuronal and glial nuclear profiles per unit area from 5 days to 24 days. This study has shown that the increase in the vascularity in the central region of the rat inferior colliculus continues for up to two weeks after the onset of hearing. By day 24 postnatally, the adult level of vascularity in the inferior colliculus has been reached, and regional differences in capillary density in the brain are evident. The decrease with age in the number of nuclear profiles per unit area is probably because of growth in the volume of the neuronal perikarya and processes, along with cell emigration reported to occur at early postnatal ages.

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List of Abbreviations

Sm	-	Small
Md	-	Medium
Lg	-	Large
Cross-sect.	-	Cross-sectional
Oblig.	-	Oblique
Long.	-	Longitudinal
L	-	Left
R	-	Right
d	-	day
vs	-	versus
N.S.D.	-	Not Statistically Different
S.D.	-	Standard Deviation
r	-	raw data analysis result
t	-	transformed analysis result
H. & E.	-	Hematoxylin and Eosin

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INTRODUCTION

The mammalian brain is dependent on a competent blood supply for its function. The development of the vascular system in the central nervous system of the rat begins early during embryonic development and continues during postnatal development (Bar: 1983). In rats (and in humans), the brain is not well vascularized at birth (Bar: 1980; Nousek-Goebel and Press: 1986). Many of the maturational changes occurring in the vascular framework happen during the first three weeks of postnatal development (Caley and Maxwell: 1970; Bar and Wolff: 1972; Bar: 1980; Rowan and Maxwell: 1981a).

Blood Vessel Development in the Rat Brain

Stages of Vascularization

There are two major stages of development of the vascular system in the brain of rats. Both stages begin in early embryogenesis, with the second stage continuing during postnatal development (Bar and Wolff: 1972; Bar: 1980).

Little work has been done with respect to the developing vascular system in the brainstem. The pattern of the formation of the vascular framework in the brainstem appears to be similar to that found in the cerebral cortex (Stoeter et al: 1980). Since detailed analysis has been applied to the cerebral cortex, this region will be used to

illustrate the stages of development.

Stage One: External Vascularization

The early cranial blood vessels arise from the aortic arch and grow towards the ventral aspect of the neural tube. There, surrounding the primitive brain and ventricles, they form a perineural vascular plexus (rabbit, Strong: 1964; rat, Bar: 1980, 1983; hamster, Marin-Padilla: 1985). From the perineural vascular plexus, the leptomeningeal vascular system arises (Bar: 1980). It is from the leptomeningeal system that the intracerebral vessel sprouts will grow (Strong: 1964; Bar and Wolff: 1972; Bar: 1980; Marin-Padilla: 1985).

Stage Two: Internal Vascularization

It is in this stage that the neural tissue itself is invaded by the primitive vascular sprouts. Stages of intracortical vascularization have been identified by different authors. The earliest stages were based on "tiers" or capillary plexuses (Strong: 1964) within the cerebral cortex. Four stages of intracortical vascularization were described by Conradi and Sourander (1980).

Stage 0 No "intra-epithelial" vessels are observed in the neural tissue.

Stage I There are straight stem vessels that have penetrated into the cortical tissue and are

branching and interconnecting in the ventricular zone.

Stage II Includes Stage I plus the formation of a vascular plexus in the more superficial part of the ventricular zone.

Stage III Includes Stage II plus additional capillary plexuses established in the intermediate zone and the subventricular zone.

The first "intra-epithelial" vessels are observed in the rat cerebral cortex on day 12 (embryonic age) (Bar and Wolff: 1972) and in the rat spinal cord on day 11 (embryonic age) (Phelps: 1972).

Vascular sprouts arise from the leptomeningeal system and approach the cortical surface. The sprouts establish contact with the external limiting membrane (a basement membrane,(Hamilton et al: 1972)) of the cortex. The sprouts penetrate the membrane and grow radially towards the ventricular zone. This penetration into the neural tissue occurs before the cortical plate is formed (Strong: 1964; Bar: 1980; Wolff: 1978; Marin-Padilla: 1985).

The vascular sprout consists of a solid cord of primitive endothelial cells of mesodermal origin. Initially, there is no lumen. There are fine filopodia and larger pseudopodia-like projections at the tip of the sprout (Bar and Wolff: 1972; Bar: 1980; Caley and Maxwell: 1970; Marin-Padilla: 1985). It has been suggested that these fine

filopodia may be "looking" for metabolic or angiogenic factors which may in turn determine the direction and growth of the new capillary (Marin-Padilla: 1985). It has also been suggested that the filopodia may be responsible for the anastomosis occurring between two vessels when contact has been made (Bar: 1980; Marin-Padilla: 1985).

The first vascular plexus to be established is found in the ventricular zone. The sprouts which had penetrated the neural tissue and grew towards the primitive ventricles are referred to as stem vessels. In the ventricular zone, the stem vessels branch, anastomosed with other branches from other stem vessels, thus forming the ventricular vascular network (Strong: 1964; Bar: 1980; Conradi and Sourander: 1980; Marin-Padilla: 1985). As growth of the cerebral cortex continues, the original stem vessels grow in length accordingly, to continue to supply the deeper cortical layers. Newly penetrating vessels will supply the superficial cortical layers (Strong: 1964; Wolff: 1978; Bar: 1980).

After birth, the continued development of the vascular system occurs primarily during the first three weeks. There are structural and functional changes in the rat central nervous system along with changes and growth of the vascular framework (Bar: 1980; Caley and Maxwell: 1970; Rowan and Maxwell: 1981a).

Some of the structural and functional changes that

occur within the rat cerebral cortex during the first three weeks of postnatal development include increases in cortical surface area and cortical thickness; and increasing metabolic demands of the neural tissue (Caley and Maxwell: 1970; Bar: 1980). There is also a qualitative metabolic change towards pathways utilizing glucose and oxygen (Betz and Goldstein: 1981).

The greatest overall increase in the cortical volume occurs during the first week to ten days of postnatal growth. Both the cortical surface area and the cortical thickness increase (Bar: 1980; Caley and Maxwell: 1970). According to the results reported by Caley and Maxwell (1970) the cortical thickness in the rat cerebral cortex increased from 0.8 mm in the newborn to 1.9 mm in the 9 day old rat. The cortical thickness was 2.1 mm in the 21 day old rat (Caley and Maxwell: 1970). Other growth changes include maturation of the neurons, axons and dendrites growing, glial proliferation and differentiation, myelination of axons, and other processes (Bar: 1980; Dardennes et al: 1984; Schonbach et al: 1968).

Changes Within the Vascular Framework

The stem vessels (those that penetrated into the cortex early) elongate proportionately to the increasing thickness of the cortex in order to continue supplying the ventricular and subventricular zones. At the same time, because of the

expanding cortical surface area, new vascular sprouts continue to arise from the leptomeningeal system and penetrate into the cortex. These new vessels do not grow deep into the cortex. They branch in the superficial layers of the cortex, forming a capillary network. New vascular sprouts are not observed penetrating the cortex after the second week of postnatal growth. The remainder of the vascular sprouts that are observed are those arising from the established intracortical vessels, predominantly the capillary segment (Caley and Maxwell: 1970; Bar: 1980, 1983; Rowan and Maxwell: 1981a).

With the cortical volume continuing to increase even after the second week of postnatal development, the density of the radial stem vessels decreases. However, the numerical density of intracortical branches per mm^3 increase during the second and third week. Maximum values were noted on Day 20 of postnatal development (Bar and Wolff: 1973 as reported in Bar: 1980).

Four "surges" or rapid increases in the number of intracortical vascular sprouts or branches in the rat cerebral cortex have been identified. These surges occurred within three time frames which are as follows:

A) From birth to Day 4, sprouts were largely found in the superficial third of the cortex.

B) From Day 7 to Day 11, with peak surges on Day 8 and 10. There was increased sprouting throughout

the cortex with consistently higher numbers found in the middle third of the cortex.

C) A final surge was found on Day 14, occurring predominantly in the middle third of the cortex (Rowan and Maxwell: 1981a).

Capillary Growth

Capillaries grow by two processes which are inter-dependent. These two processes are called sprouting (or branching) and elongation. Vascular sprouts or branches arise from existing vessels, usually the capillary segment. These sprouts consists of a solid cord of cells with pseudopodia-like projections at their tip. When contact has been made with another vessel or sprout, they coalesce and the lumen becomes patent shortly thereafter (Bar: 1980; Rowan and Maxwell: 1981b).

There are two mechanisms by which capillaries elongate. Firstly, by mitotic division of the primitive endothelial cell, followed by migration of the post-mitotic cell in a longitudinal direction (Bar: 1980). The peak of endothelial cell proliferation in the cerebral cortex occurs between five and nine days of postnatal development (Robertson et al: 1985). Secondly, the capillary elongates by the "stretching" of the endothelial cell itself (Bar: 1980). This "stretching" occurs primarily during the second and third week of postnatal development. There is an increase in the average length of the endothelial cell, a decrease in

the average thickness of the endothelial cell wall, and a decrease in the frequency of inter-endothelial contact zones in capillary cross-sections (Bar: 1980).

Ultrastructure of Central Nervous System Capillaries

Careful ultrastructural studies on developing capillaries in the rat cerebral cortex (Bar and Wolff: 1972; Bar: 1980; Rowan and Maxwell 1981a and 1981b), rat spinal cord (Phelps: 1972; Hannah and Nathaniel: 1974) and in the rat medulla (David and Nathaniel: 1981) have been completed. The basement membrane does not appear as early as the vessels do and does not fully mature until the third and fourth week of postnatal development (Bar and Wolff: 1972). Astrocytic processes complete their investment around the capillary by the end of the first week of postnatal development in the spinal cord (Hannah and Nathaniel: 1981) and by three weeks of age in the cerebral cortex (Bar and Wolff: 1972; Bar: 1980). Tight junctions between the endothelial cells are present at early stages of embryonic development (Saunders and Møllgard: 1984) but are structurally different between the fetal stage and 2 weeks postnatally (Stewart et al: 1985).

The mature capillary has tight junctions, few pinocytic vesicles, an abundance of mitochondria and no fenestrations (Brightman: 1977).

The Auditory System in Man

The cochlear division of the eighth cranial nerve bifurcates as it enters the brainstem. It enters at the pontomedullary junction. One branch synapses with the dorsal cochlear nucleus while the other branch synapses with the ventral cochlear nucleus. These nuclei are located in the dorsal pons and are tonotopically organized.

Three acoustic striae (the dorsal, the intermediate and the ventral acoustic striae) arise from the cochlear nuclei and relay information centrally and rostrally. From the superior olivary nucleus, and other nuclei, the nerve fibers can cross the mid-line to ascend in the contralateral lateral lemniscus or ascend in the ipsilateral lateral lemniscus. The fibers in the lateral lemniscus ascend through the brainstem and terminate in the nucleus of the inferior colliculus of the midbrain.

Projections from the nucleus of the inferior colliculus go via the brachium of the inferior colliculus to the medial geniculate body (a sensory nucleus of the thalamus). The fibers from the medial geniculate body form the auditory radiation and they terminate in the auditory cortex.

Tonotopic organization is maintained throughout the auditory system and there is bilateral representation of sound input from both ears in each of the inferior coll culi and in each temporal lobe (Barr and Kiernan: 1983; Fitzgerald: 1985; Carpenter: 1985)).

Anatomy of the Rat Inferior Colliculus

Location

On the posterior surface of the midbrain (the mesencephalon) are four rounded eminences referred to as the corpora quadrigemina. These eminences are divided into the superior colliculi and the inferior colliculi by a transverse groove (Snell: 1987). In the adult rat, the inferior colliculus is covered dorso-laterally by the occipital lobe and caudally by the cerebellum. Dorso-medially, the pineal gland rests on the commissural area (Faye-Lund and Osen: 1985) (Plate 1).

Development

The neurons of the inferior colliculus are "born" beginning on Day 14 (embryonic age). The earliest generated cells were found in the central nucleus. The latest generated cells were those found caudally, medially dorsally. The region where the cells were produced appeared to be dorsally located around the caudal recess of the primitive aqueduct (Altman and Bayer: 1981; Repetto-Antoine and Meininger: 1982).

Adult Structure

The inferior colliculus can be subdivided into three major parts. There are similarities in the structure of the inferior colliculus between rats (Faye-Lund: 1985), cats

(Morest and Oliver: 1984) and humans (Geneic and Morest: 1971) with minor species variation. The three major subdivisions according to the Faye-Lund and Osen (1985) study of the rat inferior colliculus are: a central nucleus, an external cortex and a dorsal cortex. Other researchers have subdivided the inferior colliculus into a central nucleus, a cortex and the pericentral nuclei (Geneic and Morest: 1971; Meininger et al: 1986; Morest and Oliver: 1984).

The central nucleus is an ovoid shaped cell mass which is located in the medial-caudal two-thirds of the inferior colliculus in the adult rat. It is covered in the ventral two-thirds by the external cortex and in the dorsal one-third by the dorsal cortex. The boundary between the two cortices is not distinct (Faye-Lund and Osen: 1985).

The Central Nucleus of the Inferior Colliculus

The central nucleus consists of two groups of neurons, divided according to morphology: the disc-shaped neurons and the multipolar or stellate neurons. The description of these two groups of neurons with their subtypes has been well documented (Geneic and Morest: 1971; Oliver and Morest: 1984; Morest and Oliver: 1984; Dardennes et al: 1984; Faye-Lund and Osen: 1985) using Golgi-impregnated tissue. The disc-shaped neurons are characterized by the positioning of their dendritic fields in columns, parallel in orientation

to each other. The stellate neurons, not as commonly found, are characterized by their dendrites extending in all directions (Faye-Lund and Osen: 1985).

In light microscopic preparations, some of the identifying features of the central nucleus in the adult rat inferior colliculus include its high cell density, little variation in cell size and a high content of myelin. Within the central nucleus, there appears to be a dorso-ventral gradient with respect to these identifying features. In the dorsal part of the nucleus, the cell size is smaller, packing density high and the myelin staining 'faintest' (Faye-Lund and Osen: 1985).

The External Cortex

The external cortex covers the central nucleus ventrally, laterally, rostrally and ventrocaudally. Three layers constitute the external cortex of the rat inferior colliculus, according to Faye-Lund and Osen (1985) from studies of Golgi-impregnated preparations. The most superficial layer, Layer I, is a fibrocellular layer containing small, scattered neurons. It is a thin layer of fibers at the surface of the cortex. The middle layer, Layer II, is composed of small to medium size cells, either bipolar or multipolar cells. They have thin dendrites which are curved and highly interwoven. The deepest layer, Layer III, consists of cells with small to large diameters. Most

commonly found were the large, multipolar neurons with several long and branching dendrites. In the rostral part of the inferior colliculus where the commissural fibers are found in the deep external cortex, there is a higher myelin content then seen in the lateral region (Faye-Lund and Osen: 1985). These commissural fibers are included by Morest and Oliver (1984) in their paracentral nuclei subdivision.

The Dorsal Cortex

The dorsal cortex covers the central nucleus in the dorsocaudal and dorsomedial region of the inferior colliculus of the rat. It also consists of three layers in the rat (Faye-Lund and Osen: 1985). In the mouse, cat and humans, the dorsal and "caudal" cortex are reported to consist of four layers (Meininger et al: 1986; Geneic and Morest: 1971; Morest and Oliver: 1984).

Layers I and II are similar in structure to those Layers I and II in the external cortex. Layer I is a thin, fibrocellular layer and Layer II, beneath, contains small to medium sized multipolar neurons. Layer III contained primarily small and medium sized neurons. There are few large multipolar neurons which are larger then those found in the central nucleus yet smaller then those identified in Layer III of the external cortex (Faye-Lund and Osen: 1985).

Physiology of the Inferior Colliculus

The general function is for binaural interaction and thus the inferior colliculus is important for sound localization. The inferior colliculus receives spatial and frequencyinformation and functions to integrate the incoming information (Thompson and Masterton: 1978; Jenkins and Masterton: 1982; Masterton and Imig: 1984; Fitzgerald: 1985; Clopton and Winfield: 1976; Clopton and Silverman: 1977; Silvermann and Clopton: 1977).

There is a tonotopic organization within all of the auditory pathway which is also maintained in the inferior colliculus in all mammals studied (Irvine: 1986). In general, in the rat, the varying sound frequencies are found in bands running the mediolateral width of the inferior colliculus. High frequencies are represented ventromedially and low frequencies are represented dorsolaterally (Clopton and Winfield: 1973; Altman and Bayer: 1981; Faye-Lund and Osen: 1985).

There is an excellent review on the anatomy and physiology of the auditory system by Irvine (1986) in his book: Progress in Sensory Physiology 7: The Auditory Brainstem.

Projections to the Rat Inferior Colliculus

The central nucleus of the inferior colliculus, rather then the external layers, receivesthe majority of the

ascending auditory input from the lower brainstem centers. The central nucleus receives both ipsilateral and contralateral projections as well as bilateral projections from the lower brainstem nuclei. Those lower brainstem nuclei projecting to the central nucleus include: the cochlear nuclei, superior olivary complex and the nucleus of the trapezoid body (Coleman and Clerici: 1987).

Transmitter Substances

There is not much known regarding which transmitter(s) are involved with respect to the inferior colliculus. It has been shown that in the gerbil, glycine receptors are present in the inferior colliculus and that the highest concentration of glycine receptors was found in the region associated with high-frequency (Sanes et al: 1987).

Rationale for Using the Rat Inferior Colliculus to Study Postnatal Development of Vascularity

1. The inferior colliculus is a sensory relay station in the auditory system of the rat. Rat pups do not respond to sound at birth (Wada: 1923). The external pinna and auditory meatus are closed at birth and do not open until approximately Day 12 of postnatal development (Crowley and Hepp-Reymond: 1966). Rats do not respond to sound until Day 9 at the earliest. But by Day 12, rats do respond to loud,

high-pitched sounds (Wada: 1923; Crowley and Hepp-Reymond: 1966). Our study of the inferior colliculus was designed to examine this region before and after the onset of hearing. The commencement of cochlear activity and input along the eight nerve, at the time of onset of hearing is assumed to be a trigger for maturational changes along the entire auditory path.

2. The functional maturation of the auditory system in rats begins during the postnatal development. Maturation begins as late as Day 10 but is established by Day 20, the time of weaning (Bosher and Warren: 1971; Crowley and Hepp-Reymond: 1966). There is both physiological and anatomical evidence that the auditory system requires balanced sensory input for its normal development and maturation (reviewed in Rubel: 1984 Ann. Rev. Physiol.). The inferior colliculus has continued neuronal structural development up to Day 20 of postnatal development (Dardennes et al: 1984; Coleman and Clerici: 1987). Binaural interaction is present in the rat inferior colliculus after Day 10 postnatally. Physiological testing shows that the critical period for establishment of binaural interaction is between Day 10 and Day 30 of postnatal development (Clopton and Silverman: 1977). Thus, although the auditory centers of rats are relatively late to mature, the period of functional maturation is approximately three weeks long.

3. It has been shown that in the adult rat, the inferior colliculus is one of the brain regions showing a high density of capillaries per unit area (Gross et al: 1987). It also has the highest rate of glucose metabolism in the brain, and within the central nucleus, there is a higher capillary density, volume fraction, and glucose metabolism as contrasted with the lateral zone of the inferior colliculus (Sokoloff et al: 1977; Sokoloff: 1980; Fenstermacher et al: 1985; Gross et al: 1987).

4. Studies have been completed on the early prenatal rat inferior colliculus (Altman and Bayer: 1981; Repetto-Antoine and Meininger: 1982). Studies have been completed on the adult rat inferior colliculus (Fenstermacher et al: 1985; Sposito et al: 1985; Gross et al: 1987; Faye-Lund and Osen: 1985). Very little is known regarding the normal pattern and time sequence of capillary growth during the first three weeks of postnatal development in the inferior colliculus of the rat.

Objectives

1. To establish the methods for tissue analysis in the central region of the inferior colliculus of the rat.
2. To analyze by several measures the changes in

vascularity in the central region of the inferior colliculus of the midbrain with age.

3. To compare left and right sides of the normal developing inferior colliculus of the midbrain in rats.
4. To examine, if any, rostro-caudal differences in the central region of the inferior colliculus in rats.

MATERIALS AND METHODS

Experimental Animals

Sprague-Dawley rat pups were used in this investigation. The pups were housed with their respective mother in standard animal care conditions. The mothers were allowed food and water ad libitum.

Four groups of three animals each were used, summarized as follows:

Group A Four to five days old; average weight was 10.67 $\pm$ 0.29 grams.

Group B Nine to ten days old; average weight was 19.33 $\pm$ 1.53 grams.

Group C Fourteen to fifteen days old; average weight was 35.20 $\pm$ 3.12 grams.

Group D Twenty-two to twenty-four days old; average weight was 71.07 $\pm$ 2.72 grams.

Animals were taken from several litters. Since the litter size varied, and that parameter affects the body weight of the pups, the pups were selected to a standardized weight as indicated above. The date of birth was taken as Day 0 of life. Both male and female pups were used, because the sex of the pups had a minimal influence on body weight before 38 days of age. (The sex of each pup was recorded, but in preliminary studies there was no evidence that sex

affects brain growth in the auditory regions of the young pups (Craigie: 1920)).

Perfusion Procedure

The animals were sacrificed at the appropriate age between 0900 and 1200 hours. The animals were anesthetized using either subcutaneous (Group A and B animals) or intraperitoneal (Group C and D animals) injection of Sodium Pentobarbital (Allen and Hanburys), (four to five milligrams per hundred grams body weight).

A mid-line abdominal incision was made to expose the diaphragm. The diaphragm was cut away from the thoracic cage, thus exposing the heart. A small incision was made at the point of the left ventricle of the heart. A blunt syringe needle was inserted and threaded up to the aorta. The right auricle was cut as the perfusion was started to allow perfusate outflow.

The syringe needle was attached to a three-way stop valve. All animals were perfused with mixed aldehydes fixative in two successive stages. There was no pre-wash.

The first stage fixative was composed of 1.0 percent paraformaldehyde and 1.25 percent glutaraldehyde in 0.08 Sorensen's phosphate buffer (pH 7.4). The second stage fixative contained 2.0 percent paraformaldehyde and 2.5 percent glutaraldehyde in 0.1 M Sorensen's phosphate buffer (pH 7.4).

The amount of fixative perfused was as follows:

Stage One: Average amount was 39.56 cc with a range of 15 cc (in young animals) to 70 cc (in the older animals). Average run time of perfusion was three minutes.

Stage Two: Average amount was 73.50 cc with a range of 25 cc (in young animals) to 110 cc (in the older animals). Average run time of perfusion was seven minutes.

The brains were dissected from the skulls immediately after perfusion was completed and re-immersed in the stage two fixative for up to one and one-half hours at room temperature. This was followed by a cold buffer wash.

Tissue Selection and Handling

Inferior Colliculus: Two transverse cuts were made through the midbrain of the brainstem to remove the inferior colliculus. One cut was made just rostral to and the second cut was made just caudal to, the inferior colliculus. Right and left halves were separated at the midline. Pieces were trimmed and placed in 1 percent osmium tetroxide (in 0.1M buffer) at room temperature. The smaller pieces (from the younger animals) were maintained in the osmium for at least one hour. The larger pieces (from the older animals) were left in the osmium for up to two and one-half hours.

Cortex: A cut was made through the brain at the level of

the optic chiasm. A coronal slice, approximately one millimeter thick, was taken from the left frontal cortex. The piece was trimmed, saving the brain region between the corpus callosum and the surface of the cortex; between the sagittal and mid-sagittal planes. The cortex pieces were also placed in 1 percent osmium tetroxide at room temperature for a minimum of one hour.

Tissue samples were dehydrated in a series of graded, ascending alcohols. The following time sequence was employed:

50% alcohol:water	10 minutes
70% alcohol:water	10 minutes
95% alcohol:water	two times at 5 minutes each.
Absolute alcohol	three times at 10 minutes each.

This was followed by three changes in propylene oxide (Fisher Scientific Company), ten minutes each.

The tissue samples were embedded in epoxy plastic JEMBED 812 from J.B.EM Services, Inc. using the following protocol:

Propylene Oxide:Epoxy	1:1	2 hours at room temperature
Propylene Oxide:Epoxy	1:3	Overnight at room temperature
Pure Epoxy		2 hours

Tissue samples were embedded in fresh plastic in "Beem" capsules and placed in a 45°C oven for overnight; samples were then placed in a 60°C oven for 24 hours.

Tissue Preparation

Light Microscopy:

Inferior Colliculus: One micron thick sections were cut on a Reichert Om U2 microtome. Sections were cut serially in the coronal plane through the entire thickness of the inferior colliculus. A section was saved every 50 microns. Sections were stained with 1 percent toluidine blue in 1 percent sodium borate. Slides were cover-slipped using Eukitt (O.Kindler).

Cortex: The left frontal cortex pieces from Group A (5 days) and Group D (24 days) were used. One micron thick sections were cut until the entire tissue block face was evident. One successive level (after skipping 50 microns) was also obtained. Sections were stained with 1 percent toluidine blue in 1 percent sodium borate. Slides were cover-slipped using Eukitt (O.Kindler).

Electron Microscopy:

Two ages were sampled, Group A (5 days) and Group C (14 days). Thin sections were cut on a Reichert OM U3 microtome using a Dupont Diamond knife. Sections were placed on copper 300 mesh grids.

Sections were stained using the following protocol:

Aqueous Uranyl Acetate 4.0 %	10 minutes
Rinsed well with double distilled water	
Lead Citrate (Reynolds: 1963)	10 minutes

Sections were viewed on the Hitachi HU 12 electron microscope operated at 75 KV.

Tissue Analysis

Inferior colliculus: Five levels were selected*such that the full thickness of the central part of the inferior colliculus would be sampled at regular intervals along the rostral-caudal extent. There was a minimum of 100 microns between levels in all ages sampled. The central region of the inferior colliculus, occupied by the central nucleus, in each of the selected levels was photographed on a Nikon Optiphot Microscope with a Nikon Microflex AFX attachment. Black and white Kodak Panatomic-x film was used. The entire central region was photographed systematically (40x objective lens, 100x negative magnification) (Plate 2).

Prints were made at a constant magnification using an Omega D-2 Enlarger and printed using a Kodak Ektamatic Processor. The final magnification of each print was x540. All prints in each level were examined to delete any overlap between fields.

Cortex: The central region between the corpus callosum and the surface of the cortex was selected. A layer 600 microns

* see page 98

inferior to the surface of the cortex was the starting point for systematic photography (approximately cortical layers IV and V; Zilles: 1985). The 40x objective lens was used and the final magnification of each print was x540.

Data Collection

I. Method of Patent Vessel Classification and Quantitation

Vessel profiles were classified according to two measurements:

1. Vessel profiles were classified into three groups based on patent internal cross-sectional diameter measured. The three groups are summarized as follows:

Small caliber vessels - this group included those vessel profiles with an internal diameter measurement of equal to or less than 10 microns. This included capillaries, pre- and post-capillary segments and small venules.

Medium caliber vessels - this group included those vessel profiles with an internal measurement of greater than 10 microns up to 20 microns in cross-section. This included larger venules and arterioles.

Larger caliber vessels - this group included those vessel profiles with an internal measurement of greater than 20 microns up to 30 microns in cross-section. Vessels larger than 30 microns were excluded from counts.

2. In each of the three groups, the vessel profiles were further classified according to their vessel profile orientation. This was determined by using the following equation:

$$\frac{\text{vessel profile longitudinal length}}{\text{vessel profile width}}$$

This yielded a ratio which was used to determine the vessel profile orientation, summarized as follows:

A: Cross-sectional vessel profiles - when the ratio was less than 3:1.

B: Oblique vessel profiles - when the ratio was between 3:1 and 5:1.

C: Longitudinal vessel profiles - when the ratio was greater than 5:1.

Vessel Quantitation

Two measures of vascularity were made for all vessel profile classifications in all fields.

1. Vascular Volume Density (see page 97)

The relative area occupied by a vascular component was determined using the Weibel method of point counting (Weibel: 1979). A trial was undertaken initially to determine the appropriate grid to be used (Appendix 1). When an intersection of the grid was found over the vessel lumen, wall or endothelial nuclear profile, it was considered a "hit" and counted. (The total number of

possible hits was 1400 or more.) The forbidden lines were respected (Plate 3 & p.116). The following formula was used to determine the vascular volume density (for each level individually):

$$\frac{\text{Number of hits over specific vessel profile type}}{\text{Total number of hits possible per level}} \times 100$$

2. Numerical Counts

Actual counts of patent blood vessel profiles in the area covered by the grid were done according to their classification. This gave two types of information.

A) The calculation of the percent of a specific blood vessel profile type of the total number of vessel profiles counted for each level individually. The following formula was used:

$$\frac{\text{Number of specific vessel profile type in given area}}{\text{Total number of vessel profiles in same given area}} \times 100$$

B) The number of specific vessel profile types in a unit area. A unit area of 1 mm^2 was established. The following formula was used for conversion of the counts of vessel profiles in given area to a number per unit area in each level.

$$\frac{\text{Number of specific vessel profile types}}{\text{Total area sampled in given level } (mm^2)}$$

II. Method of Nuclear Profile Classification and Quantitation

Nuclear profiles were classified into six categories. These included neuronal nuclear profiles, glial nuclear profiles (which included astrocytes, oligodendrocytes, and microglia), endothelial nuclear profiles, pycnotic figures and mitotic figures. An "unknown" category was also established.

Numerical Counts

The counts of the nuclear profiles according to their classification were completed in the same area as the measurements of vascularity for each individual level. This count yielded two types of information:

A) The percent of a specific nuclear profile category of the total number of nuclear profiles counted.[1] The following formula was used for this calculation:

$$\frac{\text{Number of specific nuclear profiles in given area}}{\text{Total number of nuclear profiles in same given area}} \times 100$$

B) The number of specific nuclear profiles per unit area for each individual level. A unit area of 1 mm^2 was used. The following formula was used for this calculation:

$$\frac{\text{Number of specific nuclear profiles}}{\text{Total area sampled in given level } (mm^2)}$$

---

1 This total number does not include those nuclear profiles in the endothelial nuclear profile category.

III. Neuronal Nuclear Diameter

The diameters of the neuronal nuclear profiles were estimated by two methods:

1. Area equivalent diameter method as described by Weibel was completed in two age groups (the 5 day old and 14 day old groups) (Weibel: 1979).

2. Using the Filar micrometer method of measuring nuclear diameter. This method was used only in the 14 day old sample. Both the long and short diameters of the neuronal nuclear profiles were measured and averaged. The data by filar micrometer produced similar results as by area equivalent method (Appendix 2).

Statistical Analysis (in consultation with M. Cheang, Biostatistics Unit).

The means and standard deviations used, reflect either the means of 3 rats or the means of 15 levels, (5 levels per rat) 3 rats per side, per age group. When the N value is given as 3, $\pm$ S.D., the values in the table will be the means of 3 rats (in each case the mean of a single rat is of 5 levels) plus or minus the standard deviation. These values indicate the variation between rat to rat means within one age group. To obtain those values, the Number Cruncher Statistical System (NCSS) Version 5.0 was used (Dr. Jerry L. Hintze, Utah: 1987). To complete age to age comparisons between the means, the One-Way (Unweighted)

ANOVA (NCSS) was used.

When the N value is given as 15, ± S.D., three rats were used; for each rat the 5 levels were treated as 5 replicates, and the means plus or minus the standard deviation obtained were derived from 15 values. This standard deviation reflects the intra-rat (level to level) variation and the rat to rat variation. This was considered to be a more "tight" method of assessing differences between the age groups compared (M. Cheang, Biostatistic Unit).

Measurements of Vascularity

For all of the three types of data obtained (vascular volume density, percent of specific profile type, and number of specific profiles per unit area), a two-way split plot analysis of variance (SAS Institute Inc., Cary, N.C.) was completed. This allowed for comparison of left side versus right side and for age related differences. For each rat, each level was entered as a data point because the principle of repeated measures was applied in the analysis.

Counts of Nuclear Profiles

Two statistical measurements were made. A three-way split plot analysis of variance (SAS Institute Inc., Cary, N.C.) was completed to determine rostro-caudal differences with respect to the neuronal nuclear profiles per unit area.

Secondly, a two-way split plot analysis of variance was completed to detect left side versus right side differences

and to detect age related differences with respect to nuclear profile measurements.

In the above ANOVA methods of analysis for age differences, an age-side interaction value was obtained. This interaction value indicated whether or not the changes found between the different ages were in the same direction on both the left side and the right side. If the value obtained was significant ($p<0.05$), then the change on one side found between the ages was not parallel to the changes on the other side. Therefore, analysis of differences between the age groups were completed one side at a time and secondly, the left side was not combined with the right side in the parameter measured.

The three age group comparisons made were as follows: 5 day versus 9 day; 9 day versus 14 day; and 14 day versus 24 day. Left side was compared with left side and right side was compared with right side. When multiple analysis is completed, the alpha value chosen should be divided by the number of comparisons made to obtain the correct alpha value to be used in determining significant versus non-significant differences. There were three age group comparisons made, therefore the alpha (.05) was divided by three. Values obtained with $p<0.0167$ indicated that particular comparison to be significantly different.

For each age group, the left side was compared to the right side in the same age group, generating four

comparisons. Thus the alpha (.05) was divided by four and values obtained with $p<0.0125$ indicated left-right differences to be significant within a particular age group.

Transformations

Since ratios and percentages were calculated, the raw data was transformed to common logarithms for analysis. Significant and non-significant results using both raw data analysis and transformed data analysis will be shown.

Determination of Sample Size (Sokal and Rohlf: 1969)

A "power analysis" was completed to determine appropriate sample size. Based on 3 rats per age group, the power of the study is estimated to be in excess of 95% (for the small cross-sectional blood vessel profiles per unit area). The power analysis takes into account the standard deviation within each age group and the size of the difference between age groups.

Plate 1 The location of the central nucleus at three points in the rostro-caudal extent of the inferior colliculus in a 9 day old rat. Sections shown are approximately 100 microns apart. The left hand picture is the most rostral and the right hand picture is the most caudal.

Coronal sections, right side, paraffin 5 microns thick, H. & E. Stain.

mag. X 25

CN - Central Nucleus of Inferior Colliculus
VTN - Ventral Tegmental Nucleus
Aq - Cerebral Aqueduct
P - Middle Cerebellar Peduncle
RE IC - Recess of the Inferior Colliculus
CoN - Ventral Cochlear Nucleus
V - Chief Sensory Nerve of V Nerve
DTN - Dorsal Tegmental Nucleus
IV - IV Ventricle
CBLM - Cerebellum

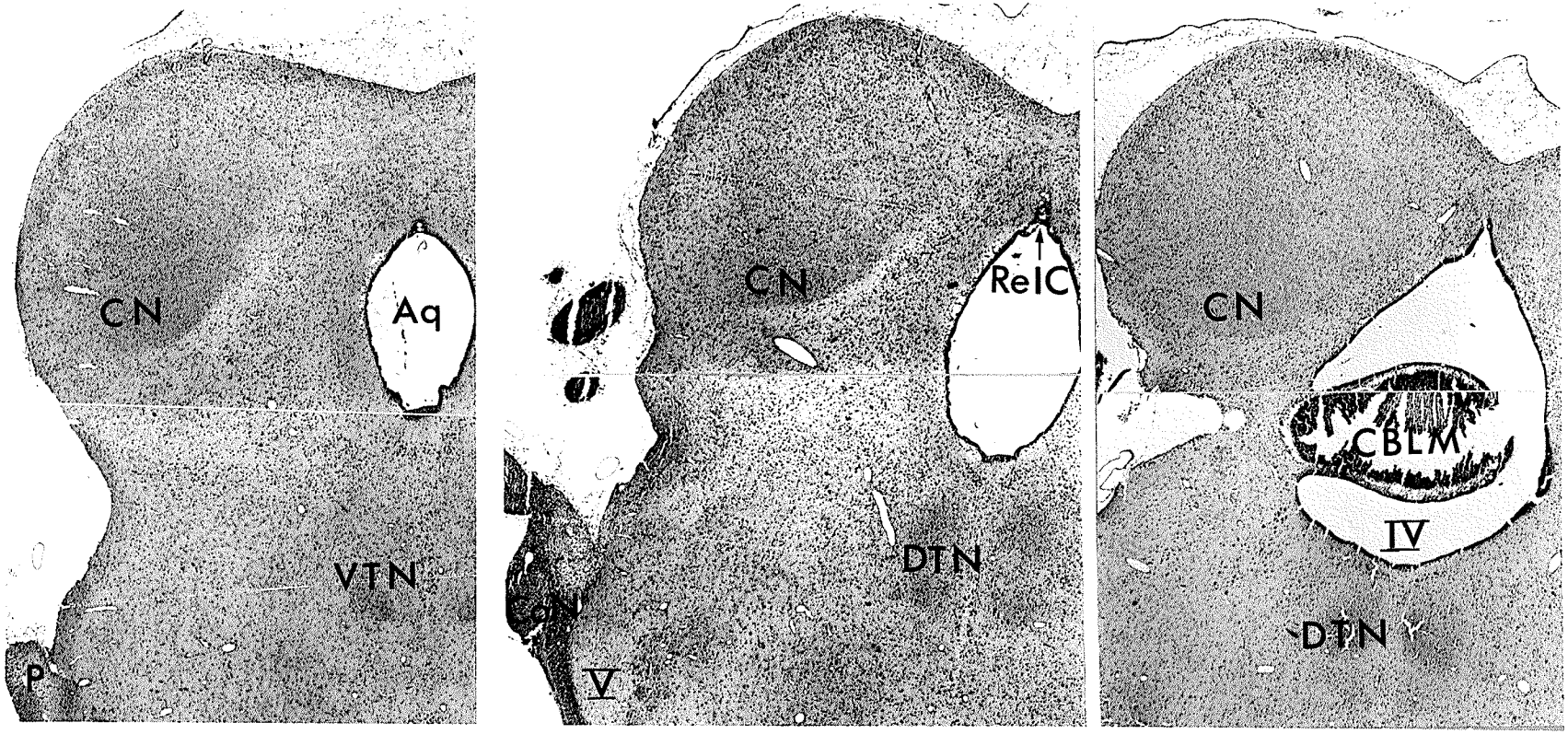

CN
Aq
VTN
P
CN
ReIC
DTN
V
CN
CBLM
IV
DTN

Plate 2 A schematic representation of the five levels sampled in the coronal plane through the rat inferior colliculus. There was a minimum of 100 microns between the individual levels chosen. Level 1 was rostral and Level 5 was caudal.

CN - Central Nucleus of Inferior Colliculus
R - Rostral end of Inferior Colliculus
C - Caudal end of Inferior Colliculus

Inferior Colliculus

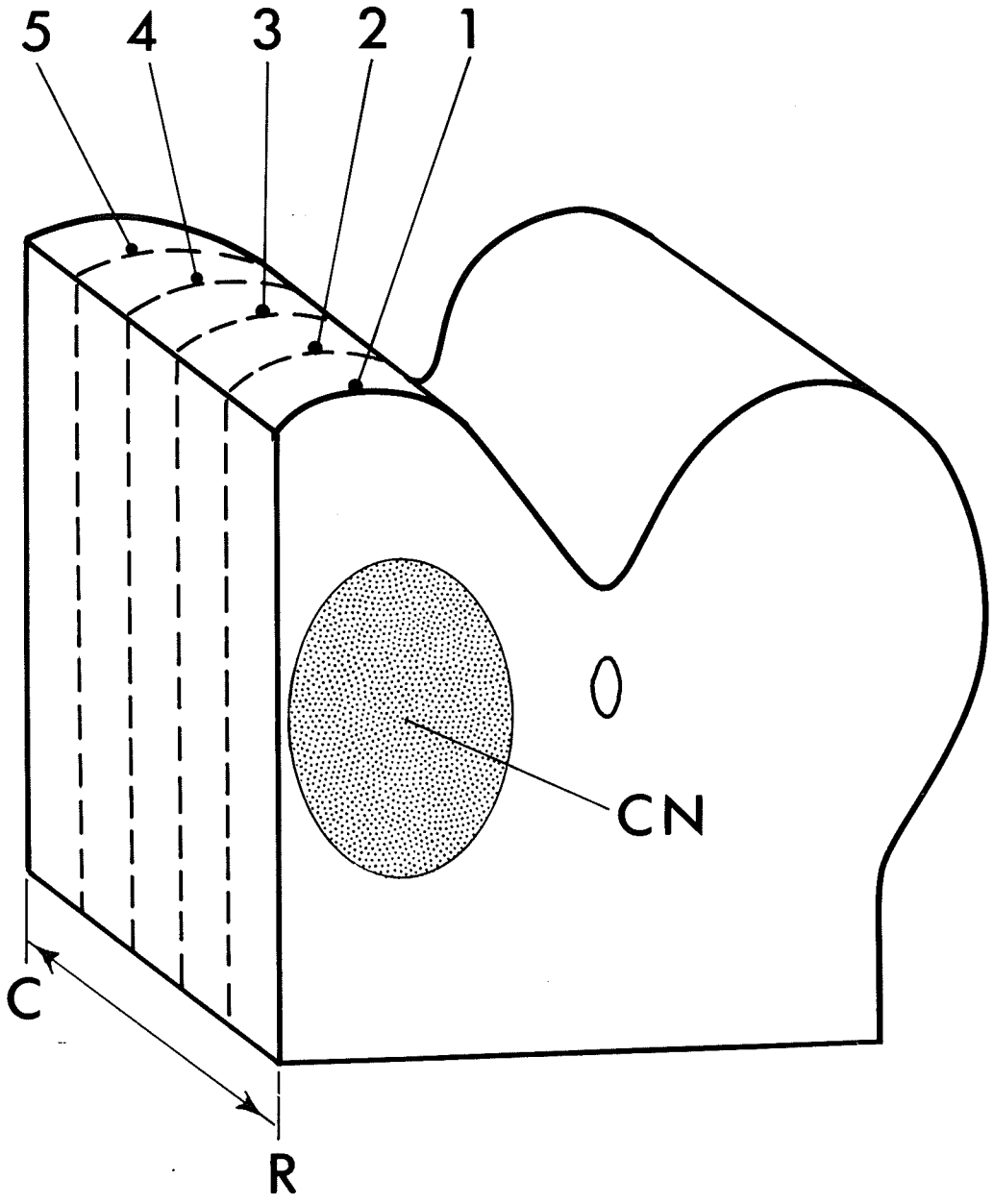


Plate 3 An example of the central nucleus of the inferior colliculus with the lattice test system superimposed. Note those blood vessel profiles touching the forbidden lines (FL); the intersections over those profiles would not be counted. The intersection over the small vessel profile "0" would be counted. Note the examples of a small cross-sectional vessel profile (S); a medium cross-sectional vessel profile (M); and, a large cross-sectional vessel profile (L). The lattice test system was adapted from page 360 of Weibel, 1979.

Nine day old, 1 micron thick sections, plastic, Toluidine Blue Stain.

mag X 540

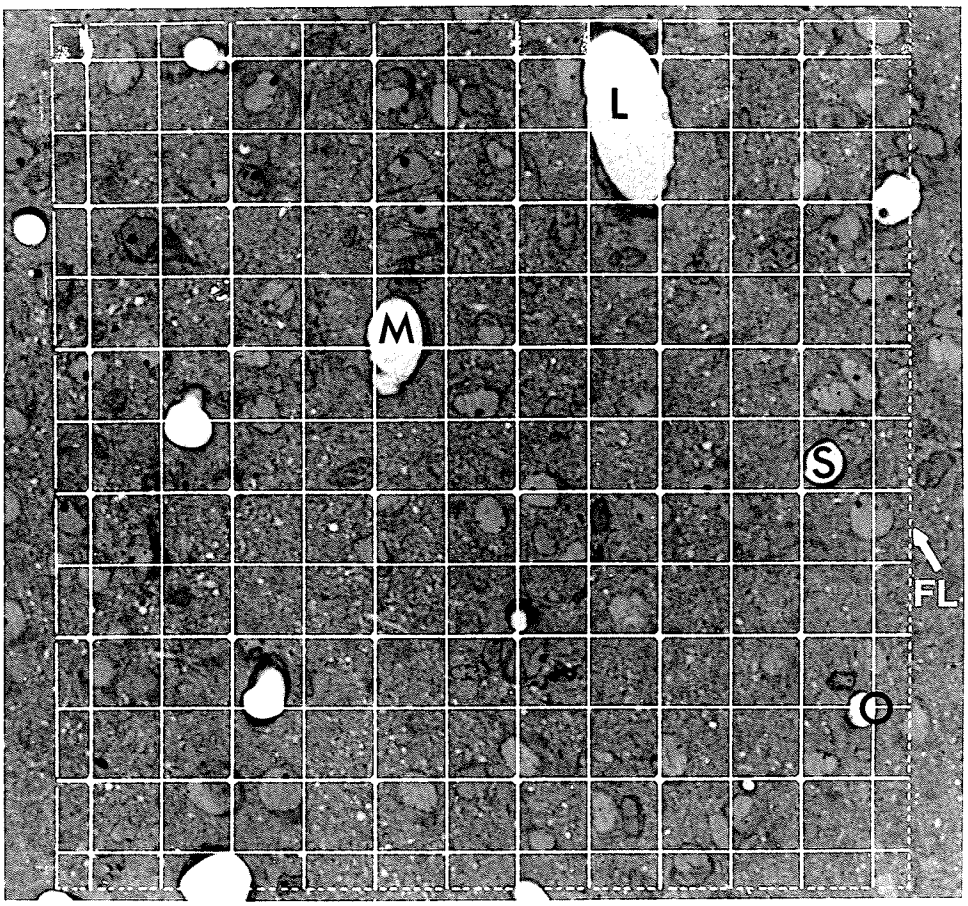

L
M
S
FL
C

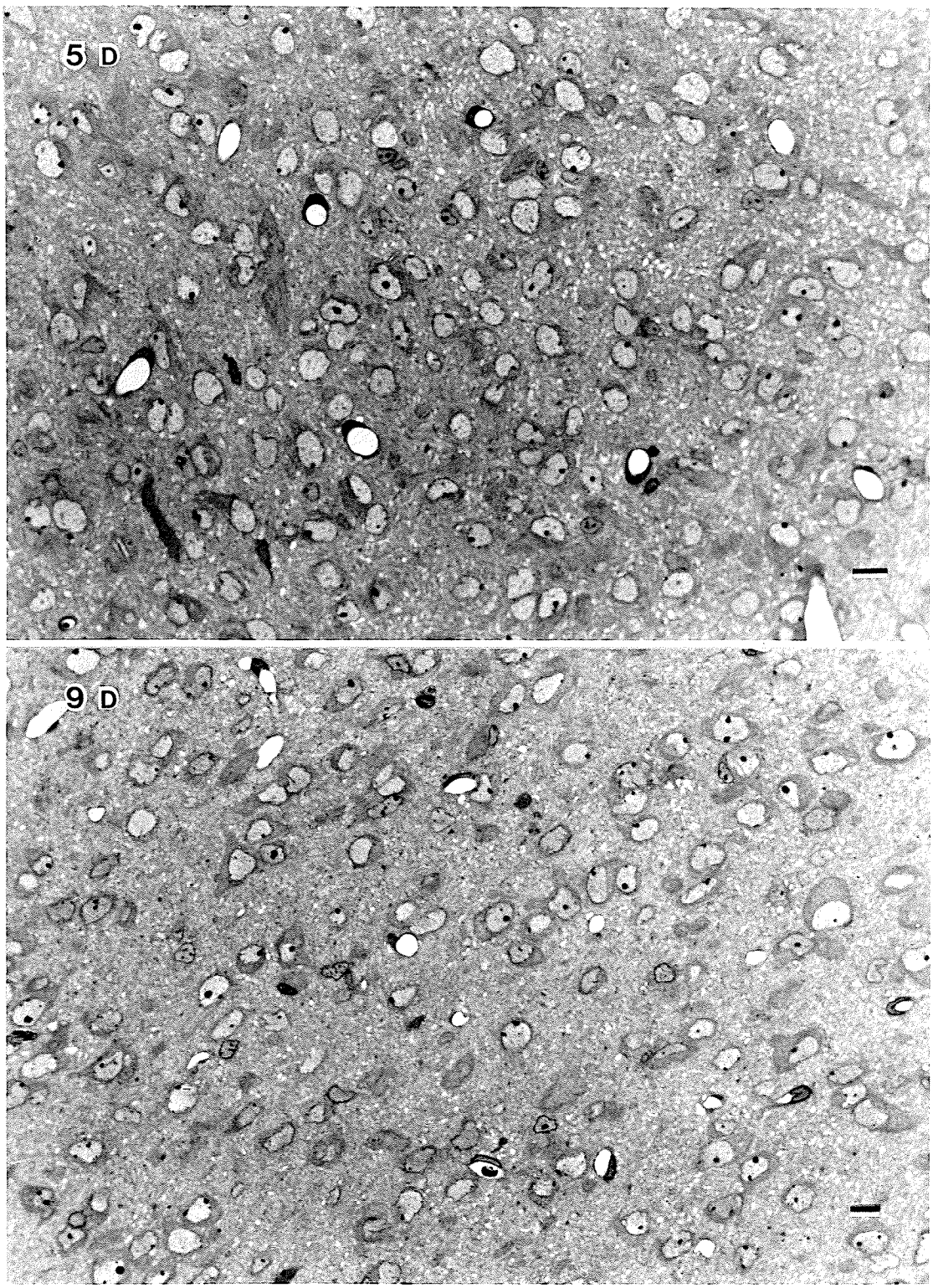
Plate 4 An example of central nucleus of the left inferior colliculus in a 5 day old rat (top) and a 9 day old rat (bottom). Note the few blood vessel profiles in the 5 day old rat with a few more vessel profiles found in the 9 day old rat. Note the closely packed nuclear profiles with minimal perikarya around the neuronal nuclear profiles and the absence of myelination.

mag X 540

Plate 5 An example of central nucleus of the left inferior colliculus in a 14 day old rat (top) and a 24 day old rat (bottom). Compare with the previous two ages and note the following; the increased number of blood vessel profiles, the nuclear profiles farther apart from one another, and the increased amount of perikarya around the neuronal nuclear profiles. Note the increased amount of myelination found in the 24 day old rat.

One micron thick plastic sections, Toludine Blue Stain. Scale Bar = 10 microns

mag X 540


5 D
9 D

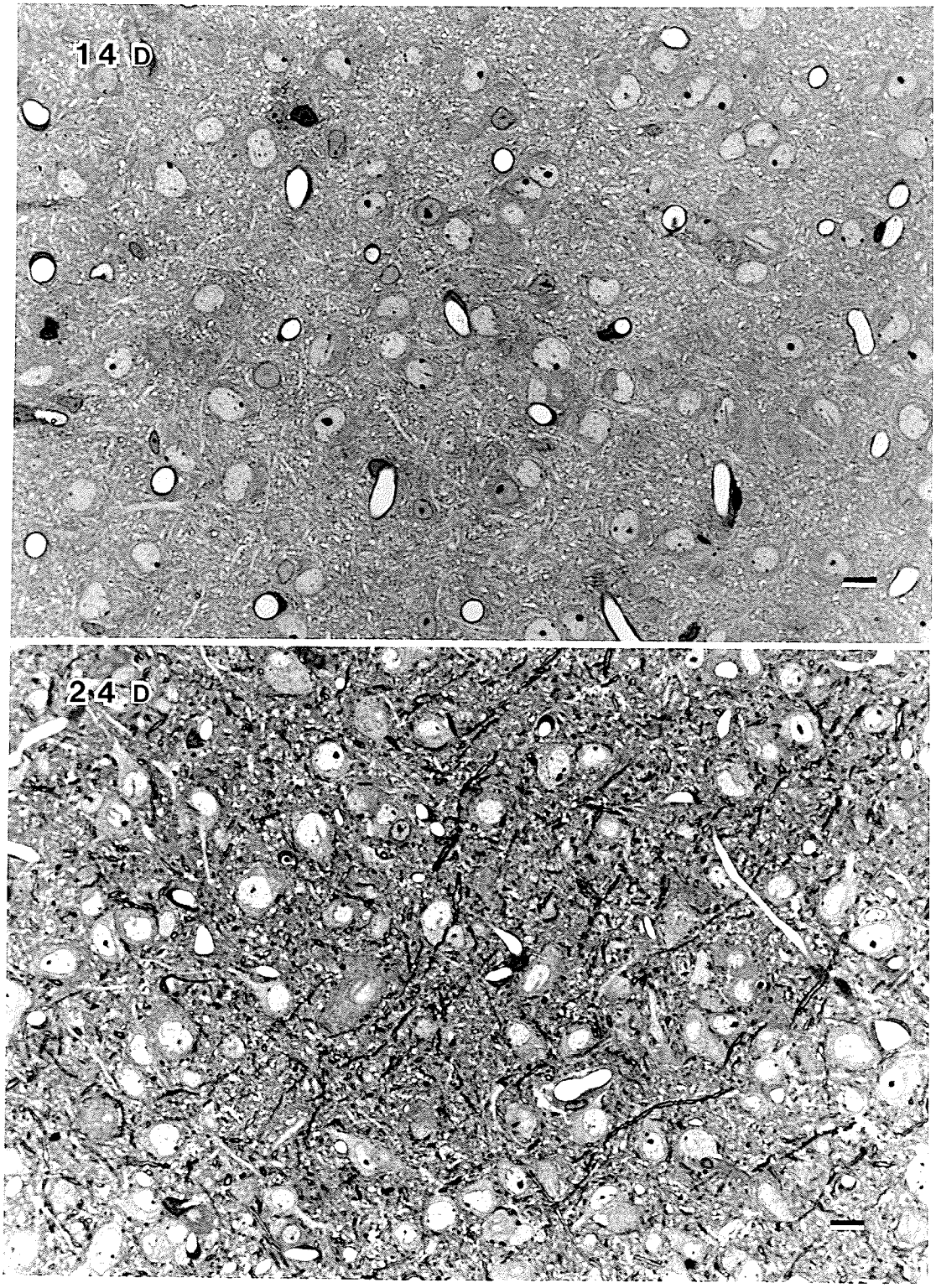

14 D
24 D

Plate 6 An example of an amoeboid microglial cell in the central nucleus of the 5 day old rat inferior colliculus. The cell contains lipid droplets (L) and lipofuscin granules (Lf) within the cytoplasm. These cells are found in normal developing brain. mag X 12000
Uranyl Acetate & Lead Stain
Scale Bar = 1 micron

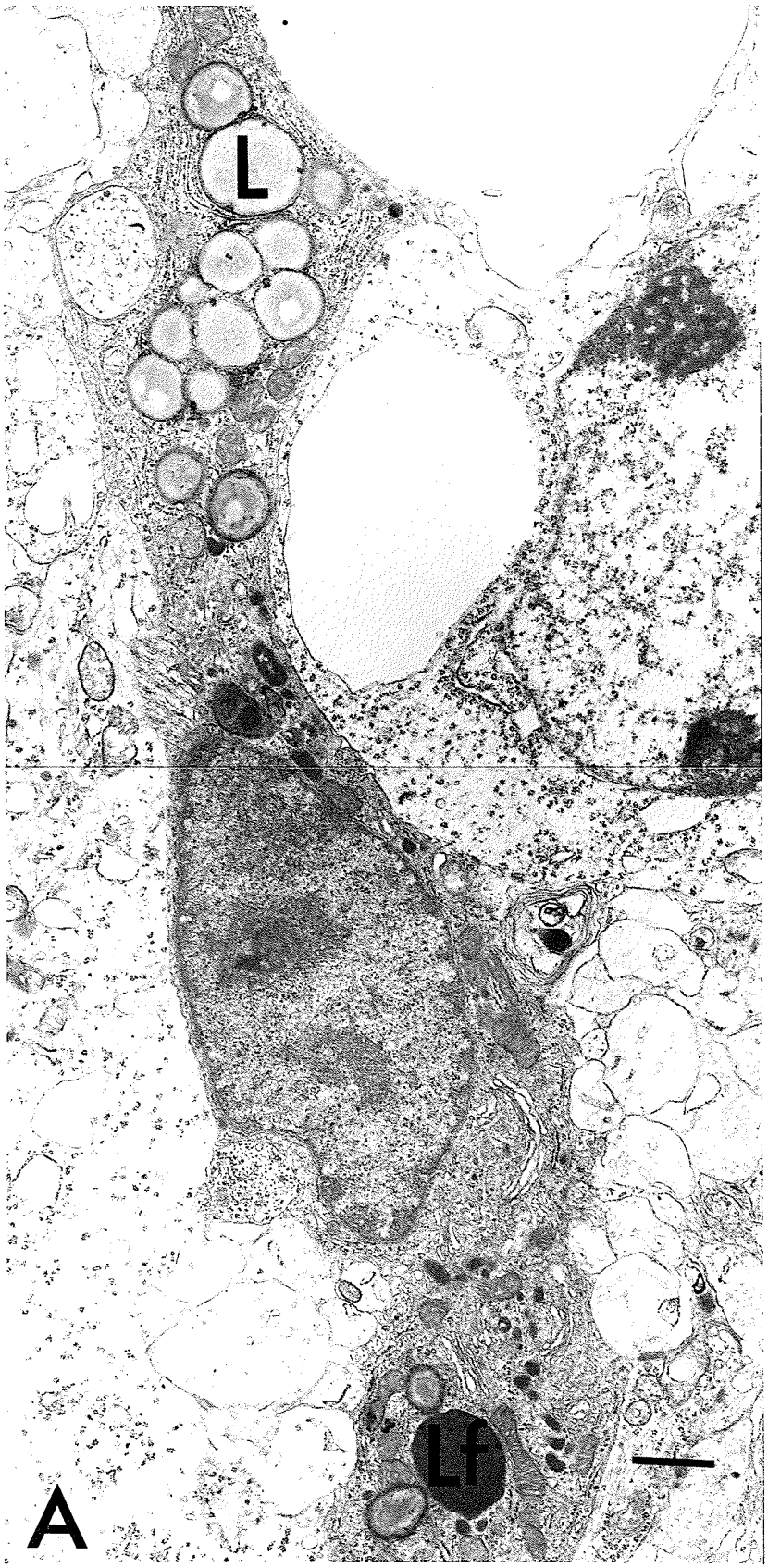

L
Lf
A

Plate 7 B,C,D Different cell profiles observed in the central nucleus of a 5 day old rat inferior colliculus. B - pycnotic cell, C - mitotic figure in metaphase with a centriole (c) and D - a mitotic figures in early prophase with astroglial-like filaments in the cytoplasm (arrowhead). Inset - a higher magnification (mag. X 30,000) of the astroglial-like filaments (dark arrowhead).

mag X 12000

Uranyl Acetate & Lead Stain

Scale Bar = 1 micron

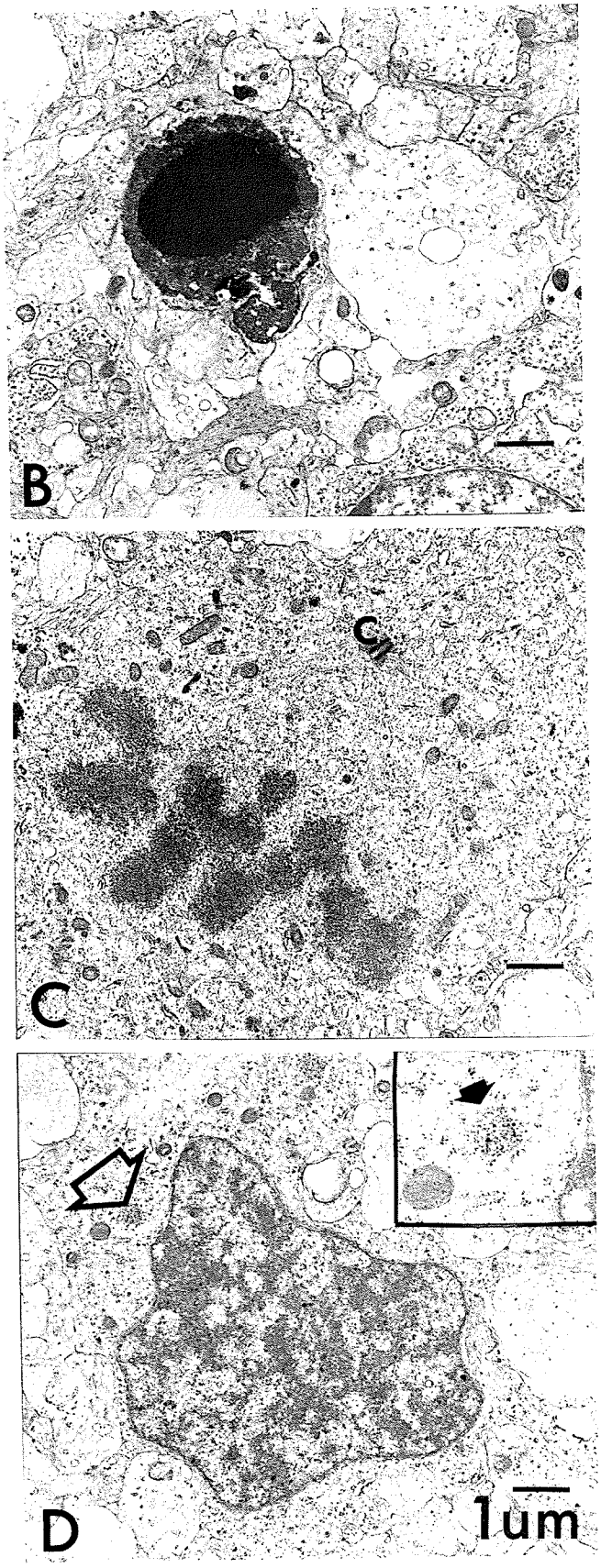
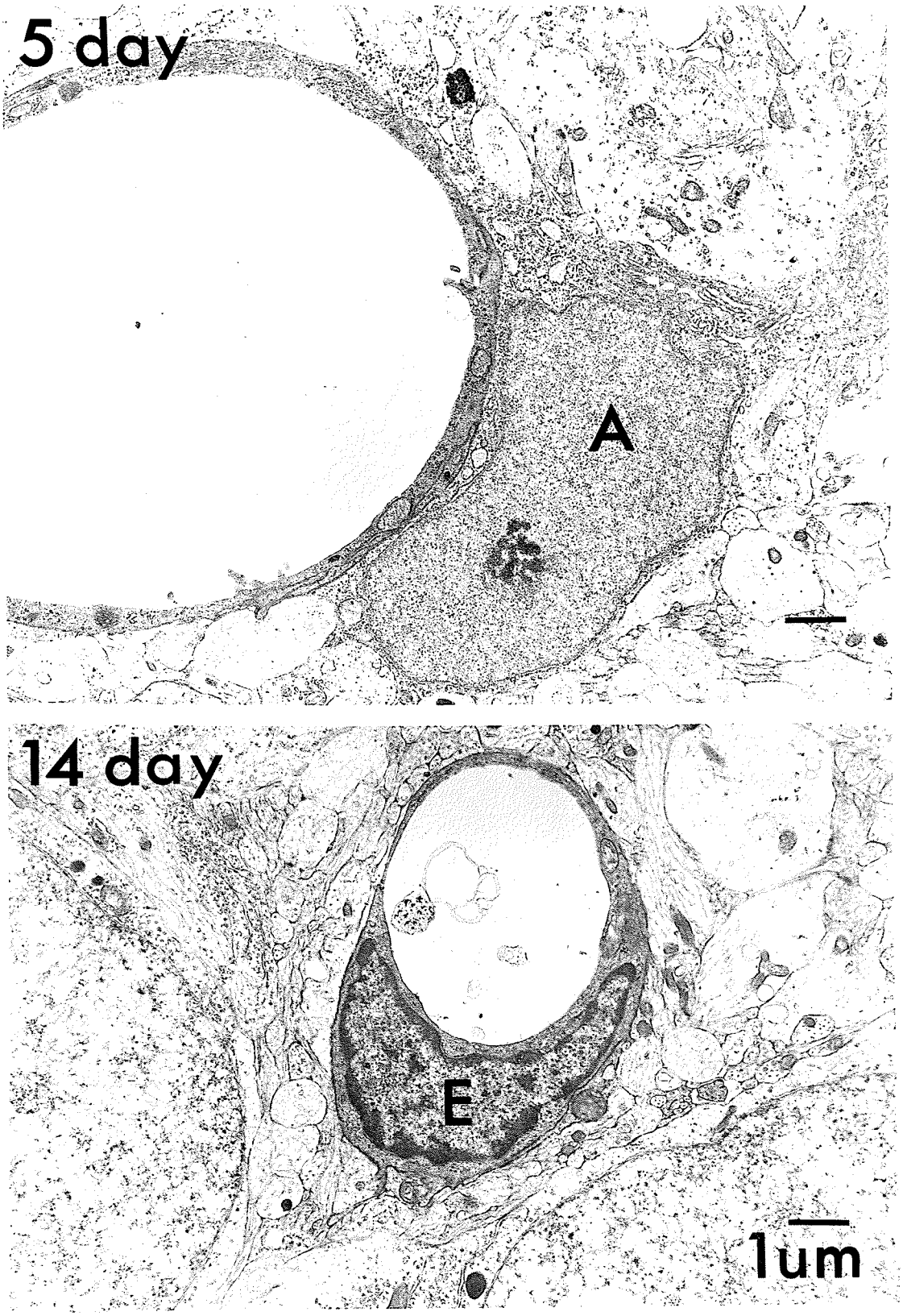

B
C
D
1um

Plate 8 Top - An astrocyte (A) in contact with part of a venule in the central nucleus of a 5 day old inferior colliculus. Diameter of vessel was approximately 10.8 microns and would be classified as a medium vessel profile.
Uranyl Acetate & Lead Stain mag. X 12000

Bottom - A capillary (internal diameter of 4.6 microns) in cross-section in the central nucleus of a 14 day old inferior colliculus. The vessel profile also contained an endothelial nucleus (E). There are parts of two neuronal nuclei surrounding the capillary.
Uranyl Acetate & Lead Stain mag X 12000


5 day
A
14 day
E
1um

RESULTS

Observations at the Light Microscopy Level (Plates3 & 4)

Some of the changes occurring with increasing age in the inferior colliculus were observed at the light microscope level. There was virtually no myelination seen in the younger two ages, the 5 day and 9 day old groups. There was some myelination observed in the 14 day old group and by 24 days old, there was a dramatic difference observed in the increased amount of myelination found. This myelination in the 24 day old inferior colliculus was seen throughout the central region of the inferior colliculus. The two fiber tracts that could be seen were the lateral lemniscal system and the commissural fibers of the inferior colliculus. These two tracts were not readily discernible in the younger age groups but were well defined in the 24 day old group.

In the younger two age groups, the neuronal nuclear profiles were closely packed together, with little cytoplasm visible In the 14 day old group, the nuclear profiles appeared more "spread" out and there were some perikarya seen around the neuronal nuclear profiles. By 24 days, the nuclear profiles were even farther apart and with much more perikarya seen around the neuronal nuclear profiles. Other types of profiles were seen such as pycnotic figures and mitotic figures in the younger age groups, specifically 5

days and 9 days old. There were very few observed in the 14 day old group and none were found in the 24 day old group.

There were few patent blood vessel profiles seen in the younger age groups, the 5 day and 9 day olds, with increased numbers observed in the older age groups. In the 5 day old group, there were some nuclear profiles observed that resembled endothelial nuclear profiles with no discernible lumen. These may have been non-patent blood vessels.

Observations at the Electron Microscopic Level

The main objective of studying a sample of 5 day old inferior colliculus and a sample of 14 day old inferior colliculus was to confirm the nuclear profile identification completed at the light microscopic level. In the 5 day old sample, pycnotic figures were seen and matched what was seen in the light microscopic preparation. Mitotic figures were also found in the 5 day old sample and they were usually glial cells. Pycnotic and mitotic figures were not found in the 14 day old sample studied. Amoeboid microglial cells were found in the 5 day old sample and these are common in normal postnatal rats (Ling et al: 1982) (Plate 6,7 and 8).

In the tissue from a 5 day old rat, large, pale nuclear profiles were occasionally found. At the electron microscopic level, they appeared to be astrocytes and thus classified as glia.

Quantitative Results

The results will be presented in the following order:

1. from the central region of the inferior colliculus
2. from the left frontal cortical region sampled
3. a comparison between the left inferior colliculus and the left frontal cortex.

For purposes of clarity, the four age groups will be referred to as 5 days, 9 days, 14 days and 24 days. The results presented will be averages (unless otherwise stated) with their standard deviation (unless otherwise stated).

I. RESULTS FROM THE INFERIOR COLLICULUS

A. AREA SAMPLED

TABLE 1

Average Area Sampled per Side per Age Group

Per Individual Level mm^2

Side	5 Days	9 Days	14 Days	24 Days
Left	0.458	0.497	0.494	0.531
S.D.	±0.077	±0.067	±0.083	±0.069
Right	0.485	0.500	0.532	0.535
S.D.	±0.091	±0.068	±0.666	±0.065

The average area sampled per level per side per rat ranged from 0.458 ± 0.077 mm^2 in the 5 day old group to 0.535 ± 0.065 mm^2 in the 24 day old group. A total of 15 levels per side (5 levels per rat) were sampled in each of the age groups. The central region of the inferior colliculus (the region sampled) corresponds mostly to the central nucleus of the inferior colliculus.

B. ANALYSIS OF THE MEASUREMENTS OF VASCULARITY

1. Percentages of Patent Vessel Profile Types

The following tables will present the results of the average percent of a vessel profile type of the total vessel profiles counted in each of the age groups. Left and right sides were not combined.

TABLE 2

Average Percent of Small Vessel Profiles of Total Profiles Counted

Age and Side		Cross-Sectional	Oblique	Longitudinal
5 days	L	68.01 ±6.60	12.02 ±3.05	6.81 ±0.37
	R	59.24 ±1.80	15.04 ±2.64	8.64 ±5.81
9 days	L	66.96 ±7.21	12.95 ±1.38	7.01 ±2.33
	R	65.82 ±4.78	13.88 ±2.27	6.17 ±1.45
14 days	L	73.29 ±3.86	11.93 ± 0.82	5.24 ±1.26
	R	73.50 ±3.63	11.74 ±2.25	5.83 ±0.83
24 days	L	77.64 ±2.01	11.78 ±0.70	6.97 ±1.03
	R	78.58 ±0.87	10.56 ±1.10	6.53 ±0.22

N=3
± S.D.

TABLE 3

Average Percent of Medium Vessel Profiles of Total Profiles Counted

Age and Side		Cross-sectional	Oblique	Longitudinal
5 days	L	10.61 ±7.61	2.40 ±2.09	0.14 ±0.25
	R	13.46 ±8.65	2.10 ±0.77	1.48 ±0.52
9 days	L	10.18 ±5.69	1.78 ±1.02	0.89 ±1.12
	R	11.26 ±5.32	1.75 ±1.32	0.55 ±0.11
14 days	L	7.79 ±1.80	1.20 ±0.85	0.26 ±0.23
	R	7.03 ±3.10	1.36 ±0.64	0.21 ±0.18
24 days	L	2.91 ±0.70	0.36 ±0.17	0.16 ±0.17
	R	3.60 ±0.27	0.51 ±0.22	0.22 ±0.11

N = 3
± S.D.

TABLE 4

Age Related Comparisons

Vessel Profile Type & Side		5d vs 9d	9d vs 14d	14d vs 24d
Sm. Cross-sectional				
Left	r	N.S.D.*	p=0.0102	N.S.D.
	t	N.S.D.	p=0.0135	N.S.D.
Right	r	p=0.0081	p=0.0009	N.S.D.
	t	p=0.0047	p=0.0015	N.S.D.
Sm. Obliq. & Long.				
Left	r	N.S.D.	N.S.D.	N.S.D.
	t	N.S.D.	N.S.D.	N.S.D.
Right	r	N.S.D.	N.S.D.	N.S.D.
	t	N.S.D.	N.S.D.	N.S.D.
Md. Cross-sectional				
Left	r	N.S.D.	N.S.D.	p=0.0007
	t	N.S.D.	N.S.D.	p=0.0001
Right	r	N.S.D.	p=0.0069	N.S.D.
	t	N.S.D.	N.S.D.	N.S.D.

* $p>0.0167$

In the small patent blood vessel profile category (those vessels with an internal diameter of equal to or less than 10 microns), the majority were found to be cross-section in orientation. This was true for all four age groups sampled. There were no age related significant differences found in the 5 day versus 9 day on the left side but there were significant differences on the right side. There were age related significant differences on both left and right sides in the 9 day versus 14 day comparison. There were no significant differences found on either side in the 14 day versus 24 day old groups.

In the small, combined oblique and longitudinal vessel profiles, there were no age-related significant differences found in the three age comparisons. When comparing the left side to the right side, significant differences were found only in the 5 day old group ($p<0.0125$) in the small, cross-sectional blood vessel profile category.

Within the medium vessel profile category (those vessels with an internal diameter greater than 10 microns up to 20 microns), the majority of the profile types seen were the cross-sectional vessel type. An analysis of variance was completed in the medium cross-sectional vessel profile category only. There were significant age related differences found when using raw data comparison for 9 day right side versus 14 day right side but this did not hold true when the transformed data was analysed. In the second

comparison, 14 day left side versus 24 day left side, the age related differences were significant using both models of comparison.

There were no left side versus right side differences found in any of the four age groups with respect to the medium cross-sectional blood vessel profile category ($p>0.0125$).

In the large vessel profile category (those vessels with an internal diameter greater than 20 microns up to 30 microns), there were very few counted. Almost all of those counted were the cross-sectional profile type. Analysis was not completed with the results from this category because at all ages they accounted for less than 0.5 percent of the total profiles counted.

The majority of the patent vessel profiles counted were found to be in the small vessel profile category in all four age groups sampled. The medium oblique and longitudinal vessel profile types, combined with all of the large vessel profiles counted, accounted for less than 4 percent of all vessel profiles counted, therefore the results from those groups will not be presented.

The remainder of the results will be presented from the following three categories in each of the age groups:

a) small, cross-sectional vessel profile type.

b) small oblique and longitudinal vessel profile types combined.

c) medium cross-sectional vessel profile type.

2. Vascular Volume Density

The following tables and accompanying graphs (Figures 1 and 2) will reflect the change in the vascular volume density with age. The results presented are the average of 15 levels (5 levels per rat). Left and right sides are not combined. The vascular volume density is expressed as a percentage (see Materials and Methods, page 26).

TABLE 5

Average Vascular Volume Density per Side per Age Group

Patent Small Cross-sectional Vessel Profiles

Side	5 Days	9 Days	14 Days	24 Days
Left	0.45 ±.21	0.86 ±.26	1.57 ±.57	2.56 ±.33
Right	0.39 ±.15	0.87 ±.20	1.45 ±.39	2.87 ±.45

N = 15
± S.D.

TABLE 5b

Age Related Differences in Small Cross-Sectional Vessel Profiles

Side		5d vs 9d	9d vs 14d	14d vs 24d
Left	r	p=0.0004	p=0.0001	p=0.0001
	t	p=0.0001	p=0.0001	p=0.0001
Right	r	p=0.0001	p=0.0001	p=0.0001
	t	p=0.0001	p=0.0001	p=0.0001

TABLE 6

Average Vascular Volume Density per Side per Age

Combined Patent Small Oblique and Longitudinal Vessel Profiles

Side	5 Days	9 Days	14 Days	24 Days
Left	0.40 ±.18	0.52 ±.24	1.15 ±.45	1.78 ±.29
Right	0.44 ±.20	0.66 ±.35	1.10 ±.21	1.82 ±.45

N = 15
± S.D.

TABLE 6b

Age Related Differences in Small Combined Vessel Profiles

Side		5d vs 9d	9d vs 14 d	14d vs 24d
Left	r	N.S.D.*	p=0.0001	p=0.0001
	t	N.S.D.	p=0.0001	p=0.0001
Right	r	N.S.D.	p=0.0002	p=0.0001
	t	N.S.D.	p=0.0001	p=0.0001

* p>0.0167

TABLE 7

Average Vascular Volume Density per Side per Age

Patent Medium Cross-sectional Vessel Profiles

Side	5 Days	9 Days	14 Days	24 Days
Left	0.22 ±.19	0.34 ±.26	0.51 ±.26	0.35 ±.12
Right	0.26 ±.26	0.35 ±.19	0.44 ±.26	0.48 ±.10

N = 15
± S.D.

TABLE 7b

Age Related Differences in Medium Cross-Sectional Vessel Profiles

Side		5d vs 9d	9d vs 14 d	14d vs 24d
Left	r	N.S.D.*	p=0.0095	p=0.0097
	t	N.S.D.	p=0.0082	N.S.D.
Right	r	N.S.D.	N.S.D.	N.S.D.
	t	N.S.D.	N.S.D.	N.S.D.

* p>0.0167

Figure 1 This graph shows the average vascular volume density for the small vessel profile category in the central nucleus of the rat inferior colliculus in the four age groups. In both left and right inferior colliculus, the cross-sectional vessel profiles and the combined oblique and longitudinal vessel profiles increase with age. The average shown is of 15 values for each side, collected from 3 animals per age group.

(In this graph, and following graphs, the horizontal axis is not drawn to time scale.)

Figure 1

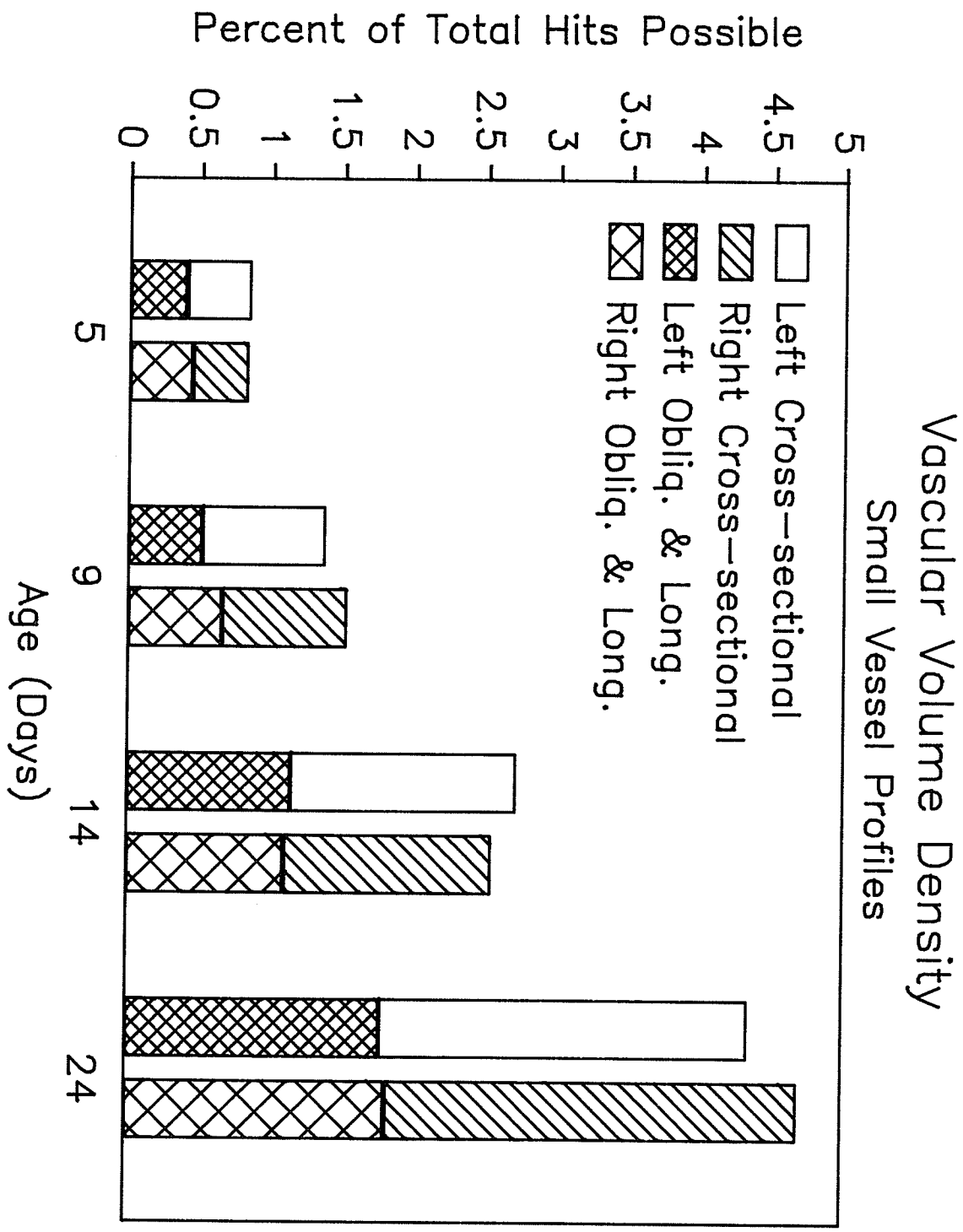
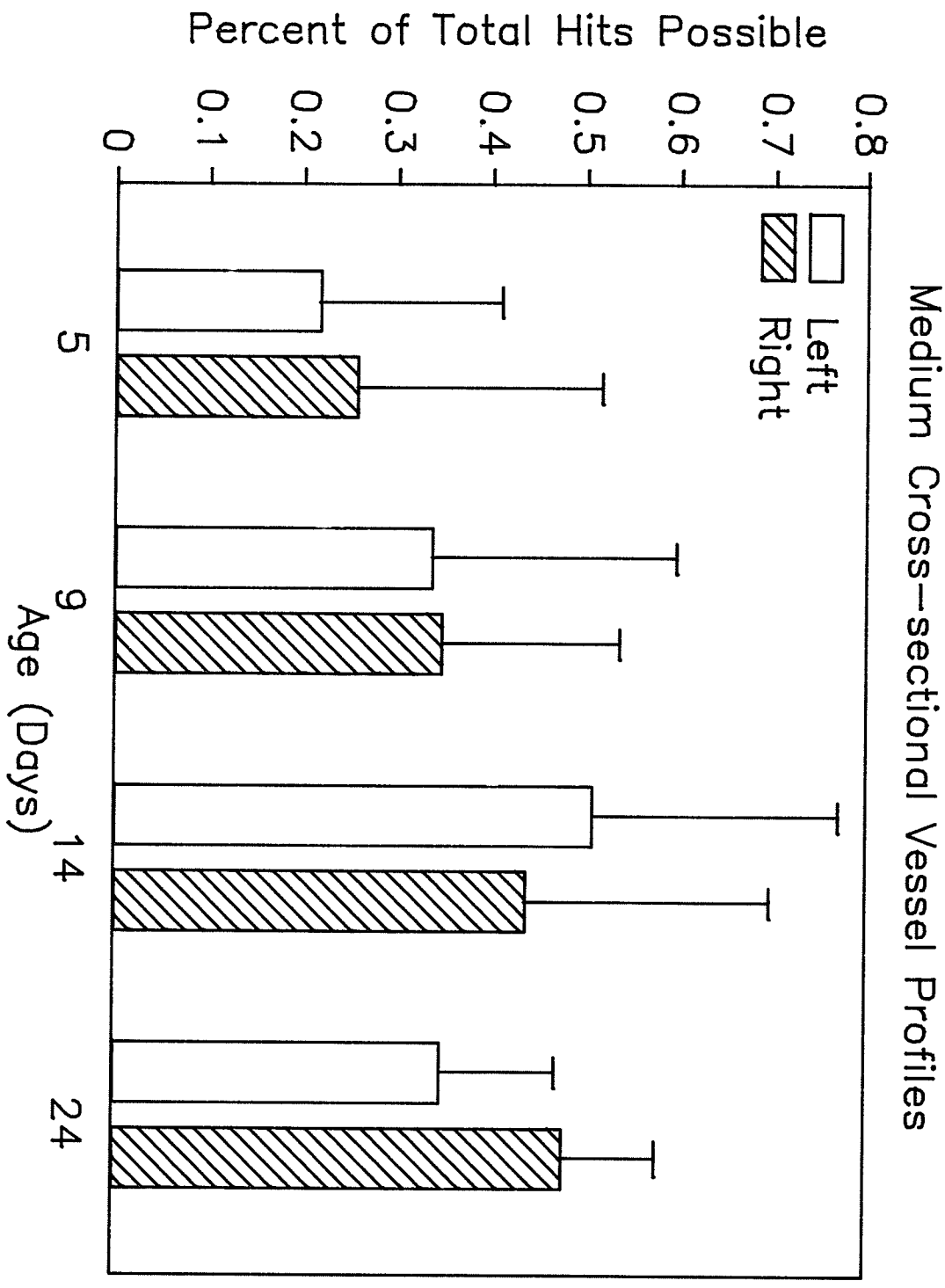

Vascular Volume Density
Small Vessel Profiles
Percent of Total Hits Possible
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
Left Cross-sectional
Right Cross-sectional
Left Obliq. & Long.
Right Obliq. & Long.
5
9
14
24
Age (Days)

Figure 2 This graph shows the average vascular volume density for the medium cross-sectional vessel profiles in the central nucleus of the rat inferior colliculus. There was some increase with age but there was much overlap between the different adjacent age groups. The values are the average of 15 levels for each side, from 3 animals per age group and standard deviations are shown.

Figure 2


Vascular Volume Density
Medium Cross-sectional Vessel Profiles
Percent of Total Hits Possible
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
Left
Right
5
9
14
24
Age (Days)

The vascular volume density of the small, cross-sectional vessel profiles increased from the 5 day old group to the 24 day old group. There were significant age related differences when analysis of variance was completed to compare the 5 day group with the 9 day group; the 9 day group with the 14 day group; and, the 14 day group with the 24 day group. This was true for all three age comparisons made.

There were no significant differences found when the left side was compared to the right side except in the 24 day old group using raw data ($p<0.0125$). (This did not occur when comparison was made using transformed data in the 24 day old group).

In the combined small vessel profile category (oblique and longitudinal vessel profiles), there was an increase in the amount of relative area occupied by a vascular component of this type. There were no age related differences found in comparing the 5 day old group with the 9 day old group (both left and right sides separately). There were age related significant differences found in the other two age comparisons; the 9 day versus the 14 day old group and the 14 day versus 24 day old group.

There were no left side versus right differences found in any of the four age groups analysed with respect to this vessel profile category ($p>0.0125$).

There was minimal change in the vascular volume density

with respect to the medium cross-sectional blood vessel profile category over age. The only significant age related differences were found to be between the 9 day left side and the 14 day left side, and in the 14 day left side versus the 24 day left side. (This second age related difference was only true when comparison was made using raw data).

There were no left side versus right side differences found in any of the four age groups sampled ($p>0.0125$).

3. Patent Blood Vessel Profiles per Unit Area

The following tables and accompanying graphs (Figures 3,4 and 5) will present the average number of patent blood vessel profiles per unit area in each of the four age groups sampled. A unit area of 1 mm^2 was used. The results presented are the averages of 15 levels (5 levels per rat). Left and right sides are not combined.

TABLE 8

Average Number of Vessel Profiles per Unit Area per Side per Age

Small Cross-sectional Vessel Profiles

Side	5 Days	9 Days	14 Days	24 Days
Left	79 ± 14	112 ± 37	247 ± 63	490 ± 42
Right	62 ± 12	111 ± 23	217 ± 37	515 ± 47

N = 15
± S.D.

TABLE 8b

Age Related Differences in Small Cross-Sectional Vessel Profiles per Unit Area

Side		5d vs 9d	9d vs 14 d	14d vs 24d
Left	r	p=0.0024	p=0.0001	p=0.0001
	t	p=0.0001	p=0.0001	p=0.0001
Right	r	p=0.0001	p=0.0001	p=0.0001
	t	p=0.0001	p=0.0001	p=0.0001

TABLE 9

Average Number of Vessel Profiles per Unit Area Per Side per Age

Combined Small Oblique and Longitudinal Vessel Profiles

Side	5 Days	9 Days	14 Days	24 Days
Left	20 ± 5	33 ± 10	59 ± 19	118 ± 19
Right	24 ± 14	34 ± 15	51 ± 8	111 ± 17

N = 15
± S.D.

TABLE 9b

Age Related Differences in Small Combined Vessel Profiles per Unit Area

Side		5d vs 9d	9d vs 14 d	14d vs 24d
Left	r	p=0.0080	p=0.0001	p=0.0001
	t	p=0.0001	p=0.0001	p=0.0001
Right	r	N.S.D.*	p=0.0006	p=0.0001
	t	p=0.0014	p=0.0001	p=0.0001

* p>0.0167

TABLE 10

Average Number of Patent Vessel Profiles per Unit Area per Side per Age

Medium Cross-sectional Vessel Profiles

Side	5 Days	9 Days	14 Days	24 Days
Left	12 ± 11	18 ± 13	28 ± 15	18 ± 6
Right	14 ± 11	19 ± 8	22 ± 13	24 ± 4

N = 15
± S.D.

TABLE 10b

Age Related Differences in Medium Cross-Sectional Vessel Profiles per Unit Area

Side		5d vs 9d	9d vs 14d	14d vs 24d
Left	r	N.S.D.*	p=0.0011	p=0.0012
	t	N.S.D.	p=0.0030	N.S.D.
Right	r	N.S.D.	N.S.D.	N.S.D.
	t	p=0.0016	N.S.D.	N.S.D.

* p>0.0167

Figure 3 The graph shows the average number of small patent cross-sectional vessel profiles per unit area in the central nucleus of the rat inferior colliculus in the four age groups sampled. The values are the average of 15 levels from 3 animals per age group, with the standard deviation. Note the increase with age. There were no differences between left and right sides.

Figure 3

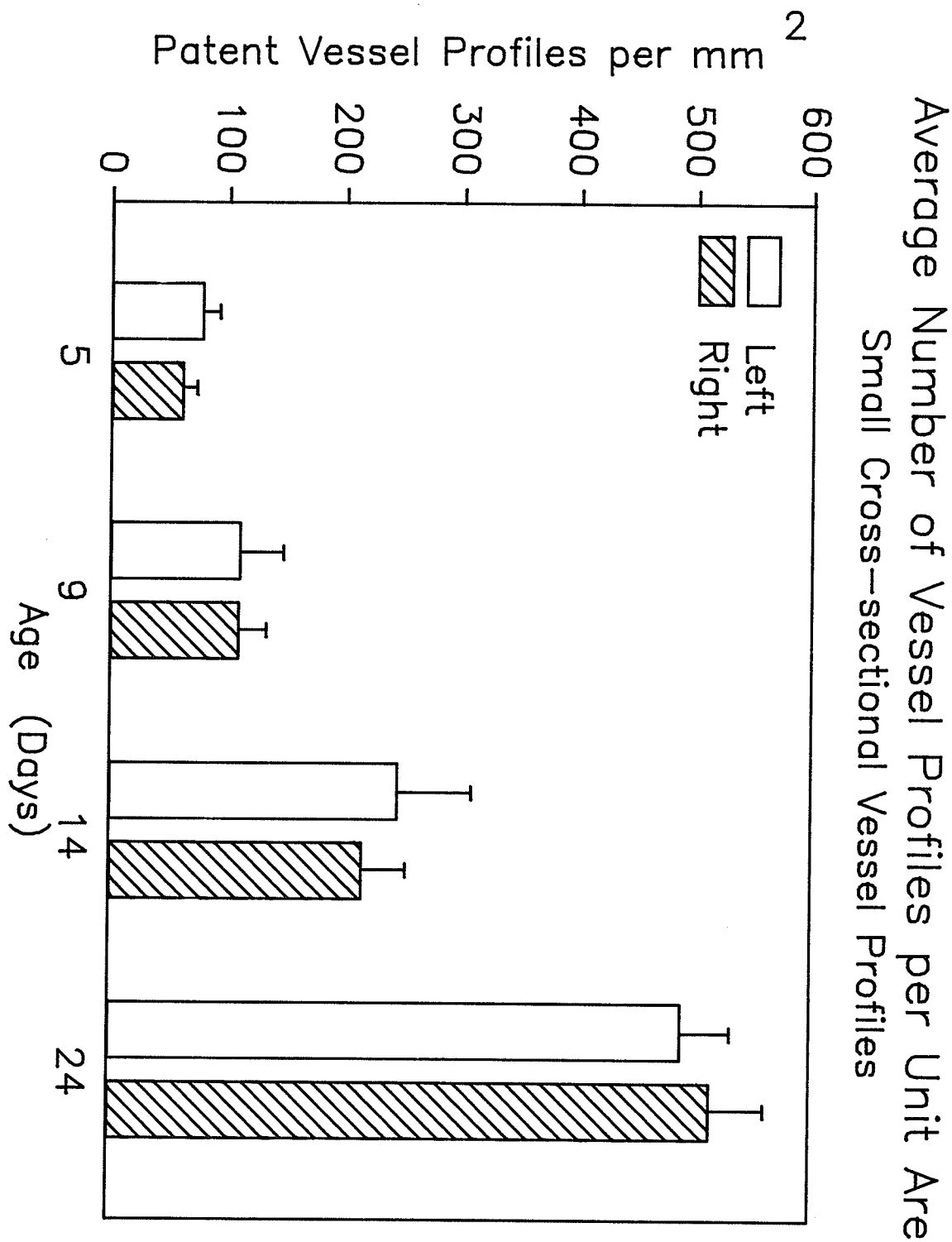

Average Number of Vessel Profiles per Unit Area
Small Cross-sectional Vessel Profiles
Patent Vessel Profiles per mm 2
0
100
200
300
400
500
600
Left
Right
5
9
14
24
Age (Days)

Figure 4 The graph shows the average number of small combined oblique and longitudinal vessel profiles per unit area in the central nucleus of the rat inferior colliculus in the four age groups sampled. The values are the averages of 15 levels, 3 animals per age group with the standard deviation. Note the increase with age. There were no differences between left and right sides.

Figure 4

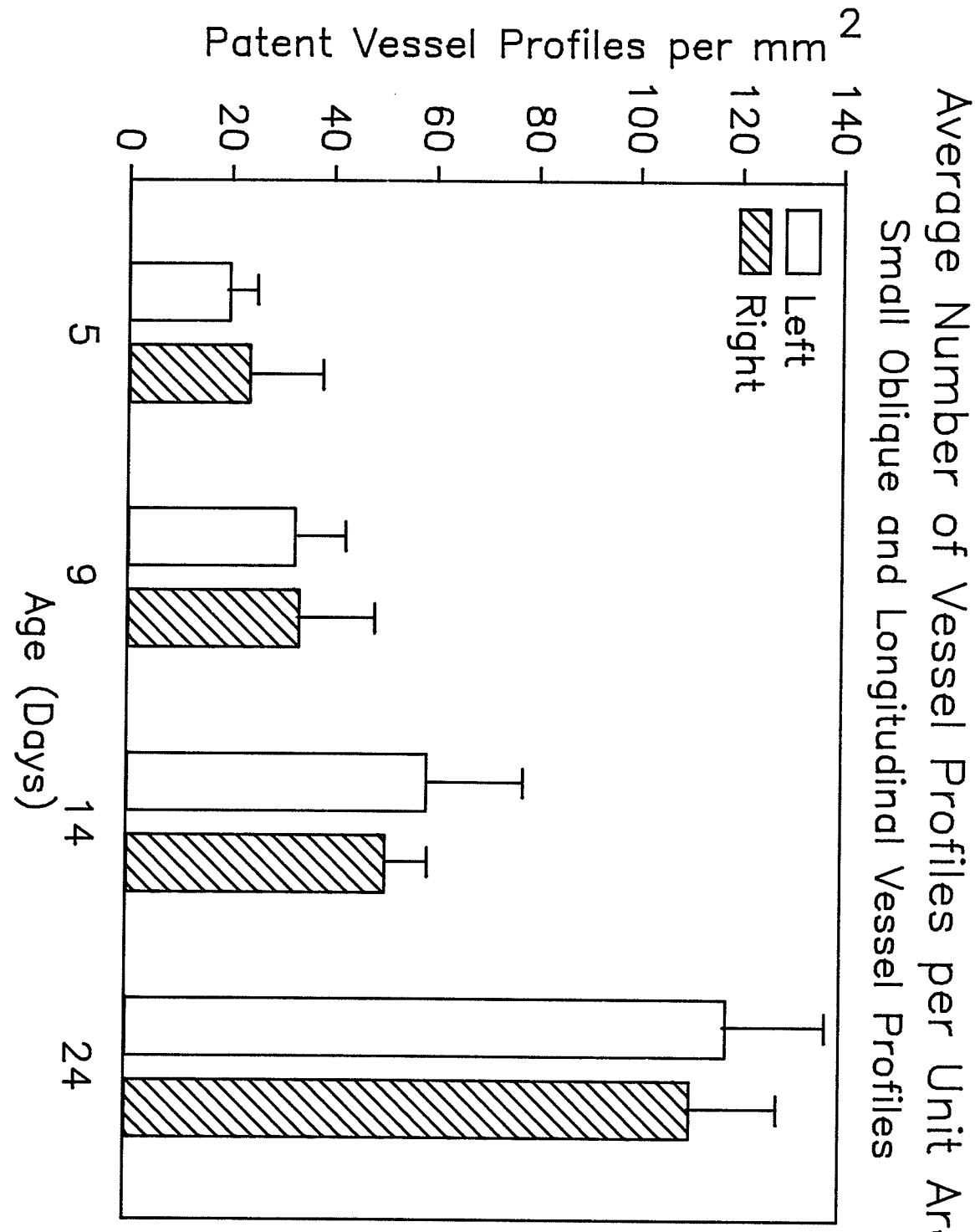
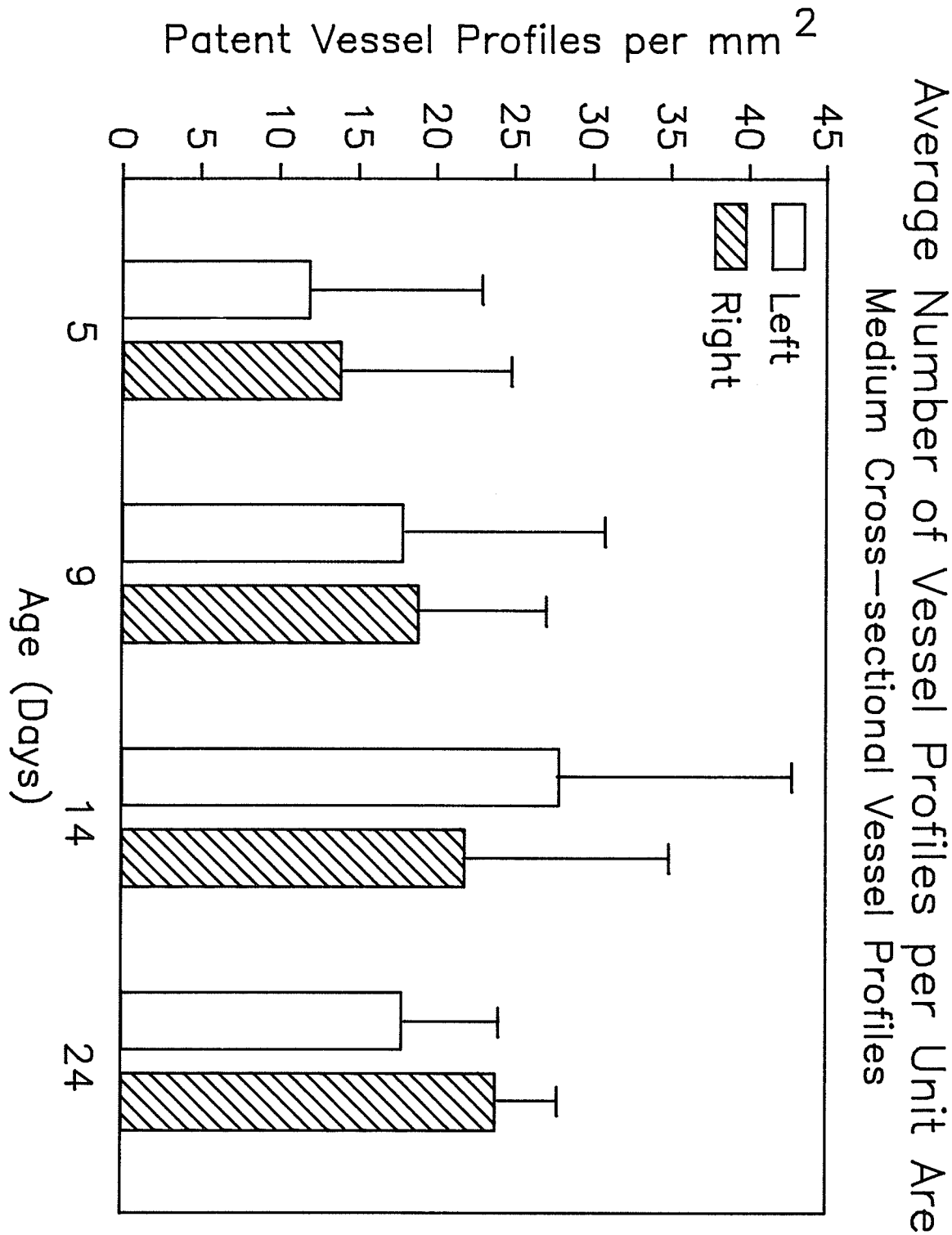

Average Number of Vessel Profiles per Unit Area
Small Oblique and Longitudinal Vessel Profiles
Patent Vessel Profiles per mm 2
0
20
40
60
80
100
120
140
Left
Right
5
9
14
24
Age (Days)

Figure 5 The graph shows the average number of medium cross-sectional vessel profiles per unit area in the central nucleus of the rat inferior colliculus in the four age groups sampled. The values are the averages of 15 levels, 3 animals per age group with the standard deviation.

Figure 5


Average Number of Vessel Profiles per Unit Area
Medium Cross-sectional Vessel Profiles
Patent Vessel Profiles per mm 2
0
5
10
15
20
25
30
35
40
45
Left
Right
5
9
14
24
Age (Days)

There was an increase in the number of patent small cross-sectional vessel profiles per unit area from the 5 day old group to the 24 day old group.

There were age related significant differences found in all three age comparisons made. This was true for both left sides and right sides analysed separately.

There were no left side versus right differences found in any of the four age groups sampled ($p>0.0125$).

There was an increase in the number of small oblique and longitudinal vessel profiles from the 5 day old group to the 24 day old group. There were age related significant differences found between the 5 day old group and the 9 day old group on the left side, using the raw data analysis. When the comparison was made using the transformed data, there were significant differences on both the left and right sides. There were significant differences found in comparing the 9 day old with the 14 day old; the 14 day old with the 24 day old group, on both left and right sides.

There were no significant differences when comparing the left side with the right side in any of the age groups sampled ($p>0.0125$).

There was only a slight increase in the number of medium cross-sectional vessel profiles per unit area from the 5 day old group to the 24 day old group. The differences found between the 5 day old and 9 day old were not significant on the left side. On the right side,

significant differences were found only after raw data was transformed and analysed. Significant age related differences were found between 9 day left side and 14 day left side only. Differences were found to be significant between the 14 day left side and 24 day left side using raw data comparison only. Other comparisons were not significant age related differences.

There were no left side versus right side differences in any of the age groups sampled ($p>0.0125$).

4. Patent Vessel Profiles Containing an Endothelial Nuclear Profile

Those vessels which had an endothelial nuclear profile in cross-section were counted. No attempt was made to categorize those vessel types containing an endothelial nuclear profile.

TABLE 11

Average Percent of Vessel Profiles with Endothelial Nuclear Profile

Side	5 Days	9 Days	14 Days	24 Days
Left	61.54	49.81	42.94	25.61
S.D.	± 3.18	± 5.47	± 6.24	± 3.83
Right	58.10	51.04	44.18	23.07
S.D.	± 1.08	± 6.30	± 5.38	± 4.41

N = 3
± S.D.

TABLE 12

Average Number of Vessel Profiles with an Endothelial Nuclear Profile Per Unit Area

Side	5 Days	9 Days	14 Days	24 Days
Left	67 ± 2	83 ± 14	142 ± 12	161 ± 22
Right	61 ± 4	85 ± 15	128 ± 6	150 ± 29

N = 3
± S.D.

In the two younger age groups, at least half of the patent vessel profiles counted contained an endothelial nuclear profile. By 24 days of age, only a quarter of the vessel profiles counted contained an endothelial nuclear profile.

There was an increase in the number of vessel profiles containing an endothelial nuclear profile per unit area from 5 days to 24 days old. There were age related significant differences found between the 5 day old right side and the 9 day old right ($p<0.05$) but not on the left side in this age group comparison. There were age related significant differences found between the 9 day old and 14 day old group on both left and right sides ($p<0.05$). There were no significant differences found between the 14 day old group and the 24 day old group.

There were no left side versus right differences in any of the four age groups studied.

C. ANALYSIS OF THE NUCLEAR PROFILE COUNTS

1. Percentages of Nuclear Profile Type

The following two tables will present the average percent of a specific nuclear profile type of the total number of nuclear profiles counted on the left and right sides. This number does not include the endothelial nuclear profiles.

TABLE 13

Average Percent of Nuclear Profile Type in each Age Group

Left Side

Nuclear Profile Type	5 Days	9 Days	14 Days	24 Days
Neuronal	71.9 ±6.08	62.0 ±6.36	60.5 ±0.86	51.7 ±5.34
Glia	26.6 ±6.19	37.1 ±7.17	37.7 ±3.00	47.8 ±4.85
Pycnotic	0.3 ±0.16	0.16 ±.02	.007 ±.012	0.00
Mitotic	0.2 ±0.30	0.05 ±.04	.007 ±.012	0.00
Unknown	0.6 ±0.51	0.7 ±0.77	0.5 ±0.14	0.5 ±0.76

N = 3
± S.D.

TABLE 14

Average Percent of Nuclear Profile Type in each Age Group

Right Side

Nuclear Profile Type	5 Days	9 Days	14 Days	24 Days
Neuronal	74.3 ±0.98	60.2 ±5.05	63.4 ±3.31	54.2 ±6.52
Glia	24.5 ±0.55	38.8 ±6.16	36.3 ±3.37	45.3 ±6.24
Pycnotic	0.4 ±0.16	0.2 ±0.08	0.05 ±0.07	0.00
Mitotic	0.04 ±0.02	0.02 ±0.02	0.03 ±.01	0.00
Unknown	0.4 ±0.50	0.8 ±1.06	0.2 ±0.09	0.5 ±0.57

N = 3
± S.D.

TABLE 14b

Age Related Differences in the Percent Neuronal and Glial Nuclear Profile Categories

Nuclear Profile Type and Side		5d vs 9d	9d vs 14d	14d vs 24d
Neuronal				
Left	r	p=0.0001	N.S.D.*	p=0.0001
	t	p=0.0001	N.S.D.	p=0.0001
Right	r	p=0.0001	N.S.D.	p=0.0001
	t	p=0.0001	N.S.D.	p=0.0001
Glial				
Left	r	p=0.0001	N.S.D.	p=0.0001
	t	p=0.0001	N.S.D.	p=0.0001
Right	r	p=0.0001	N.S.D.	p=0.0001
	t	p=0.0001	N.S.D.	p=0.0001

* $p>0.0167$

The majority of the nuclear profile types counted were neuronal nuclear profiles. This was true for all of the four age groups studied but there was a decrease in the percentages from the 5 day old group to the 24 day old group. The second largest category was the glial nuclear profile category which increased in percentage from the 5 day old group to the 24 day old group.

There were age related significant differences found between the 5 day old group and the 9 day old group in both the left and right sides. Similarly, there were age related significant differences found between the 14 day old group and the 24 day old group in both sides. However, there were no differences found between the middle two age groups, the 9 day versus the 14 day old group. This pattern was true in both of the two major categories, the neuronal nuclear profiles and the glial nuclear profiles. There were no left side versus right side differences in either of these two categories in any of the age groups studied ($p>0.0125$).

The change in percentages of the other nuclear profile types (pycnotic and mitotic figures) were not analysed statistically. There was a decrease in the percentage from 5 days to 14 days in both categories, with none being found in the 24 day old group.

2. Nuclear Profiles per Unit Area

A unit area of 1 mm^2 was used for each of the profile

categories analysed. All four categories were analysed for age-related differences. In the neuronal nuclear profile category, each level was analysed for level to level differences, left level versus corresponding right level differences, and age to age differences. In the glia profile category, left and right sides were combined; level to level and age to age comparisons were made.

(i) Neuronal Nuclear Profiles per Unit Area

a. Level to Level Differences

Level to level differences were found in the 5 day and 9 day old groups. There were no level to level differences in the 14 day old or the 24 day old group within the inferior colliculus.

Table Fifteen will present where the significant differences were found in the 5 day old group. Level One was the most rostral level and Level Five, the most caudal level sampled.

TABLE 15

AVERAGE NUMBER OF NEURONAL NUCLEAR PROFILES PER UNIT AREA PER LEVEL

LEFT SIDE

Level	5 Days	9 Days
1	1713	890
2	1949	964
3	2287	1071
4	2506	1132
5	2662	1066

N=3

RIGHT SIDE

Level	5 Days	9 Days
1	1471	776
2	1720	879
3	2210	974
4	2551	1174
5	2656	1243

N=3

TABLE 15a

AVERAGE NUMBER OF NEURONAL NUCLEAR PROFILES PER UNIT AREA

Side	14 Days	24 Days
Left	948	385
Right	923	423

N=15

TABLE 15b

Level to Level Differences in the Neuronal Nuclear Profiles per Unit Area

Five Day Old Group

Level	Left Side	Level	Right Side
1		1	
	N.S.D.		p=0.0104
2		2	
	p=0.0008		p=0.0001
3		3	
	N.S.D.		p=0.0007
4		4	
	N.S.D.		N.S.D.
5		5	
	p=0.0001		p=0.0001
	p=0.0003		

In the left side, Level One and Two were the same, but both were different from Level Three. Level Three and Four were the same and Level Four and Five were the same, but Level Three and Five were significantly different. However, on the right side, Level One and Two were different as well as being different from Level Three. Level Three and Four were different but Level Four and Five were not different.

The average number of neuronal nuclear profiles in

Level One was 1713 per mm^2 on the left side and 1471 per mm^2 on the right side. This average number of neuronal nuclear profiles per unit area increased to 2662 per mm^2 (left side) and 2656 per mm^2 (right side) in Level Five in the 5 day old group.

In the 9 day old group, there were no level to level differences found on the left side. On the right side, Level One, Two and Three were similar but Level One was different from Level Four and Level Five ($p=0.0001$). Level Four and Five were not significantly different.

The average number of neuronal nuclear profiles per unit area in Level One was 890 per mm^2 on the left side and 776 per mm^2 on the right side in the 9 day old group. The average number in Level Five on the left side was 1066 per mm^2 and 1243 per mm^2 on the right side.

b. Left Versus Right Differences

There were no left side versus right side differences found in each level. This was true in all four of the age groups studied ($p>0.0125$).

c. Age Comparison, Left and Right Sides Separately

In both the left side and the right side, there were significant differences found between the 5 day old group and the 9 day old group ($p<0.005$). This was true for all five levels compared.

In the 9 day left side compared to the 14 day left side, there were no significant differences found in any of the five levels. On the right side there were significant differences found between the 9 day and 14 day in Levels One, Four and Five ($p<0.005$). In levels Two and Three, there were no significant differences found.

In the third age group comparison, between the 14 day old group and the 24 day old group, there were significant differences found on both the left side and the right side, in all five levels ($p<0.001$). The following table will present the average number of neuronal nuclear profiles per unit area for each side. This average can be calculated because there were no level differences found.

TABLE 16

Average Neuronal Nuclear Profiles per Unit Area

Side	14 Days	24 Days
Left	948	385
Right	923	423

N = 15

The average number of neuronal nuclear profiles per mm^2 decreased from 14 days to 24 days in both the left and right sides.

(ii) Glial Nuclear Profiles per Unit Area

a. Level to Level Differences

Right and left sides were combined together in the analysis of level differences with respect to the glial nuclear profiles per unit area.

Level differences were found only in the 5 day and 9 day old groups. In the 5 day old group, there were no differences found between adjacent levels (e.g. Level One and Two; Level Two and Three; and so on). There were differences found between Level One and Five and Level Two and Five ($p<0.01$). The average number of glia per unit area in Level One was 669 per mm^2 and in Level Five it was 881 per mm^2.

In the 9 day old group, the only level difference found to be significant was between Level One and Level Five ($p<0.01$). In Level One, there were an average of 517 nuclear profiles per unit area. This increased to 687 glia nuclear profiles per unit area in Level Five.

There were no level differences found in the 14 day old or in the 24 day old groups.

b. Age Comparison, Left and Right Sides Separately

Table Seventeen will present the average number of glial nuclear profiles per unit area. The values reflect the average of the 15 levels per side per age group.

TABLE 17

Average Number of Glial Nuclear Profiles per Unit Area

Side	5 Days	9 Days	14 Days	24 Days
Left	825	597	585	341
Right	688	638	528	346

N = 15

TABLE 18

Age Related Differences in the Glial Nuclear Profiles per Unit Area

Side		5d vs 9d	9d vs 14d	14d vs 24d
Left	r	p=0.0001	N.S.D.	p=0.0001
	t	p=0.0001	N.S.D.	p=0.0001
Right	r	N.S.D.*	p=0.0065	p=0.0001
	t	N.S.D.	p=0.0019	p=0.0001

* p>0.0167

There were left versus right differences found only in the 5 day old group when all the levels on each side were averaged ($p<0.01$). The number of glial nuclear profiles per unit area showsa tendency to decrease from 5 days to 24 days.

On the left side, there were significant differences found between the 5 day and 9 day old groups and between the 14 day old and 24 day old groups. On the right side, significant differences were found between the 9 day and 14 day old groups and also between the 14 day and 24 day old groups.

(iii) Mitotic and Pycnotic Figures per Unit Area

There were few mitotic and pycnotic figures found. They accounted for less than 1 percent of total nuclear profiles counted. They were found predominantly in the 5 day old group.

The following table will present the average number of mitotic figures per unit area in each age group. Left and right sides were not combined.

TABLE 19

Average Number of Mitotic Figures per Unit Area

Side	5 Days	9 Days	14 Days	24 Days
Left	2.2	0.8	0.13	0.0
Right	1.3	0.3	0.4	0.0

N = 15

TABLE 20

Age Related Differences for Mitotic Figures per Unit Area

Side	5d vs 9d	9d vs 14d	14d vs 24d
Left	p=0.0047	not evaluable	not evaluable
Right	N.S.D.*	not evaluable	not evaluable

* p>0.0167

There were very few mitotic figures that were found in any of the age groups sampled. For statistical analysis to compare differences between the age groups, the transformed data was used.

The 5 day versus 9 day old age group comparison was the only comparison that could be completed. Significant differences were only found in the left side and not on the right side. There were no left versus right side

differences found to be significant.

The following table will present the average number of pycnotic figures per unit area. The average will be of 15 values.

TABLE 21

Average Number of Pycnotic Figures per Unit Area

Side	5 Days	9 Days	14 Days	24 Days
Left	9.87	2.67	0.067	0.00
Right	10.60	2.40	0.8	0.00

TABLE 22

Age Related Differences for Pycnotic Figures per Unit Area

Side	5d vs 9d	9d vs 14d	14d vs 24d
Left	p=0.0004	p=0.0001	not evaluable
Right	p=0.0001	p=0.0103	not evaluable

There were a greater number of pycnotic figures counted in the 5 day old group than the other three ages sampled. For statistical analysis to be completed, the transformed data was used. The data in the 14 day versus 24 day old group could not be analysed. There were significant age related differences found between the 5 day and the 9 day old group and between the 9 day and 14 day old group. This was found in both the left side and the right side in both age comparisons made. There were no left versus right side differences.

II. RESULTS FROM THE LEFT FRONTAL CORTEX

A. AREA SAMPLED

As indicated previously, two age groups were sampled; 5 day olds and 24 day olds. There were three animals per group. The following table presents the average area sampled in the two groups.

TABLE 23

Average Area Sampled

Left Frontal Cortex

Age	Area (mm^2)
5 days	0.630 $\pm$.06
24 days	0.678 $\pm$.08

B. ANALYSIS OF THE MEASUREMENTS OF VASCULARITY

1. Percentage of Patent Vessel Profile Type

The following table will present the results of the average percent of a specific patent vessel profile type of the total number of patent vessel profiles counted.

TABLE 24

Average Percent of Patent Vessel Profiles

Left Frontal Cortex

Vessel Profile Type	5 Days	24 Days
Sm. Cross-Sect.	61.06 ±5.07	78.78 ±1.24
Sm. Oblique	14.32 ±3.60	11.99 ±1.32
Sm. Longitudinal	10.18 ±3.94	7.75 ±0.28
Md. Cross-Sect.	9.29 ±8.29	2.37 ±0.54
Md. Oblique	2.54 ±2.25	0.70 ±0.38
Md. Longitudinal	2.14 ±2.58	0.00
Lg. Cross-Sect.	0.00	0.33 ±0.32
Lg. Oblique	0.48 ±0.83	0.00
Lg. Longitudinal	0.00	0.00

N = 3
± S.D.

The majority of the patent blood vessel profiles counted in the left frontal cortex in both the 5 day old group and the 24 day old group were small blood vessel profiles. Within this category, the common profile seen was the cross-sectional blood vessel profile. There were significant differences, age related, between the 5 day old cortex and the 24 day old cortex in the small cross-sectional vessel profile category ($p<0.05$).

To maintain consistency in analysis, the small oblique and longitudinal vessel profiles were combined into one subgroup. There were significant differences between the 5 day cortex and the 24 day old cortex ($p<0.05$).

There was no age related significant difference found when the comparison was made in the medium cross-sectional vessel profiles (using raw data). There were significant differences found when the comparison was made using the transformed data ($p<0.05$).

The remainder of the vessel profile types (specifically, the medium oblique and longitudinal vessel profiles and the large vessel profile category) comprised less than 6 percent of the total number of vessel profiles counted in the sample; further results from those categories will not be presented.

2. Vascular Volume Density

The following table will present the average vascular volume density in the left frontal cortex in both the 5 day old group and the 24 day old group. The values presented reflect the average of three animals with their standard deviations, and are expressed as a percent.

TABLE 25

Average Vascular Volume Density

Left Frontal Cortex

Vessel Profile Type	5 Days	24 Days	p Value
Sm. Cross-sectional	0.51 ±.13	1.67 ±.04	r 0.0002 t 0.0016
Sm. Obliq. & Long.	0.41 ±.13	1.04 ±.19	r 0.0107 t 0.0037
Md. Cross-sectional	0.19 ±.07	0.23 ±.02	r N.S.D.* t N.S.D.

N = 3
± S.D.
* p>0.05

There were age related significant differences found between the two age groups with respect to the small cross-sectional vessel profiles and the small combined oblique and longitudinal vessel profiles. There were no age-related significant differences found with respect to the medium cross-sectional vessel profile category.

3. Patent Blood Vessel Profiles per Unit Area

The following table will present the results from the conversion of the number of vessel profiles in a given area to the number of profiles per unit area. A unit area of 1 mm^2 was used. The numbers reflect the average of three animals with their standard deviation.

TABLE 26

Average Number of Patent Vessel Profiles per Unit Area

Left Frontal Cortex

Vessel Profile Type	5 Days	24 Days	p Value
Sm. Cross-sectional	53 ± 8	346 ± 44	r 0.0006 t 0.0001
Sm. Obliq. & Long.	20 ± 1	77 ± 3	r 0.0001 t 0.0001
Md. Cross-sectional	9 ± 9	11 ± 4	r N.S.D.* t N.S.D.

N = 3
± S.D.
* p>0.05

There was an increase in the small vessel profile category, in both sub-categories, from 5 days to the 24 day old group. The increase was more dramatic with respect to the small cross-sectional vessel profiles. There were age related significant differences in both of the small cross-sectional and small oblique and longitudinal vessel profile categories between the 5 day and 24 day old groups.

There were fewer medium cross-sectional vessel profiles per unit area found and there were no age related significant differences found.

III. COMPARISON BETWEEN LEFT FRONTAL CORTEX AND LEFT INFERIOR COLLICULUS IN THE FIVE AND TWENTY-FOUR DAY OLD GROUPS.

In this section, the results from the 5 day old left inferior colliculus will be compared with the 5 day old left frontal cortex. Similarily, the 24 day old left inferior colliculus will be compared with the 24 day old left frontal cortex.

A. Five Day Old Group

1. Percentage of Patent Vessel Profile Type

The following table will present the average percent of a specific patent vessel profile type found in the cortex and the inferior colliculus in this group.

TABLE 27

Average Percent of a Specific Vessel Profile Type

Five Day Old Group

Vessel Profile Type	Inferior Colliculus	Cortex	p Value
Sm. Cross-sectional	68.01 ±6.60	61.06 ±5.07	r N.S.D.* t N.S.D.
Sm. Obliq. & Long.	18.23 ±3.36	24.49 ±6.58	r 0.0349 t 0.0335
Md. Cross-sectional	10.61 ±7.61	9.29 ±8.29	r N.S.D. t N.S.D.

N = 3
± S.D.
* $p>0.05$

The majority of the specific vessel profiles found were small, cross-sectional vessel profiles. There were no significant differences found in the small cross-sectional and medium cross-sectional vessel profile categories compared between the cortex and the inferior colliculus in the 5 day old group. There were significant differences found between the two brain regions with respect to the small combined oblique and longitudinal vessel profiles.

2. Vascular Volume Density

This next table will present the average vascular volume density (expressed as a percent) in the three profile categories in the left cortex and inferior colliculus.

TABLE 28

Average Vascular Volume Density

Five Day Old Group

Vessel Profile Type	Inferior Colliculus	Cortex	p Value
Sm. Cross-sectional	0.44 ±0.17	0.51 ±0.13	r N.S.D.* t N.S.D.
Sm. Obliq. & Long.	0.40 ±0.12	0.41 ±0.13	r N.S.D. t N.S.D.
Md. Cross-sectional	0.20 ±0.16	0.19 ±0.07	r N.S.D. t N.S.D.

N = 3
± S.D.
* p>0.05

The vascular volume densities in all of the vessel profile categories remained essentially the same in the inferior colliculus and the frontal cortex. There were no significant differences found between the two regions sampled in the 5 day old group. This was true in all of the categories.

3. Blood Vessel Profiles per Unit Area

In the following table, the average number of specific vessel profiles per unit area in both brain regions sampled will be presented. A unit area of 1 mm^2 was used. The numbers reflect the average of the three animals in the 5 day old group with the standard deviation.

TABLE 29

Average Number of Patent Blood Vessel Profiles per Unit Area

Five Day Old Group

Vessel Profile Type	Inferior Colliculus	Cortex	p Value
Sm. Cross-sectional	74 ± 5	53 ± 8	r N.S.D.* t 0.0365
Sm. Obliq. & Long.	20 ± 3	20 ± 1	r N.S.D. t N.S.D.
Md. Cross-sectional	12 ± 9	9 ± 9	r N.S.D. t N.S.D.

N=3
± S.D.
* p>0.05

There were slightly more small cross-sectional vessel profiles found in the left inferior colliculus than in the left frontal cortex. The differences were significant when transformed data was compared between the two regions. When comparison made with raw data, no significant differences were found.

With respect to the combined small oblique and longitudinal vessel profiles and the medium cross-sectional vessel profiles, there were no differences found between the two regions sampled in the 5 day old group.

B. TWENTY-FOUR DAY OLD GROUP

1. Percentage of Patent Vessel Profile Type

The following table will present the average percent of a specific patent vessel profile type found in both the left inferior colliculus and the left frontal cortex. The average is of three animals with its standard deviation.

TABLE 30

Average Percent of a Specific Vessel Profile Type

Twenty-four Day Old Group

Vessel Profile Type	Inferior Colliculus	Cortex	p Value
Sm. Cross-sectional	74.64 ±2.01	78.78 ±1.24	r N.S.D.* t N.S.D.
Sm. Obliq. & Long.	18.75 ±1.71	17.74 ±1.35	r N.S.D. t N.S.D.
Md. Cross-sectional	2.91 ±0.70	2.37 ±0.54	r N.S.D. t N.S.D.

N = 3
± S.D.
* p>0.05

The majority of the vessel types found were the small cross-sectional vessel profiles. There were no significant differences found in any of the vessel profile categories when comparing the results from the left inferior colliculus with the left frontal cortex in this age group.

2. Vascular Density

Table Thirty-One will present the vascular volume density obtained in the left inferior colliculus and left frontal cortex. The values reflect the average of the three animals with their standard deviation, and are expressed as a percent.

TABLE 31

Average Vascular Volume Density

Twenty-four Day Old Group

Vessel Profile Type	Inferior Colliculus	Cortex	p Value
Sm. Cross-sectional	2.56 ±0.13	1.67 ±0.04	r 0.0006 t N.S.D.*
Sm. Obliq. & Long.	1.76 ±0.13	1.04 ±0.19	r 0.0069 t 0.0283
Md. Cross-sectional	0.35 ±0.06	0.23 ±0.02	r N.S.D. t N.S.D.

N = 3
± S.D.
* p>0.05

There was a higher vascular volume density in the 24 day old left inferior colliculus than found in the left frontal cortex in the small vessel category (both the cross-sectional and the combined oblique and longitudinal vessel profiles). In other words, there was a larger relative area of the inferior colliculus occupied by a small caliber vascular component than found in the cortex in the same age group. These differences were significant in both types of comparisons made except for the transformed data analysis in the small, cross-sectional vessel profile category.

In the medium cross-sectional vessel profile category, there was a smaller area occupied by this vessel type in both the inferior colliculus and cortex in this age group. The differences between the two regions were not significant with respect to this vessel profile type.

3. Blood Vessel Profiles per Unit Area

In the next table, the average number of a specific patent vessel profile type per unit area in both brain regions sampled will be presented. A unit area of 1 mm^2 is used. The numbers reflect the average of three animals in the 24 day old group with their standard deviations.

TABLE 32

Average Number of Patent Blood Vessel Profiles per Unit Area Twenty-Four Day Old Group

Vessel Profile Type	Inferior Colliculus	Cortex	p Value
Sm. Cross-sectional	490 ± 29	346 ± 44	r 0.0082 t 0.0314
Sm. Obliq. & Long.	118 ± 9	77 ± 3	r 0.0004 t 0.0022
Md. Cross-sectional	18 ± 4	11 ± 4	r N.S.D.* t N.S.D.

N = 3
± S.D.
* $p>0.05$

There were consistently higher numbers of small vessel profiles per unit area found in the 24 day old left inferior colliculus than found in the left cortical region sampled. These differences were significant in both the small cross-sectional vessel profiles and in the small combined oblique and longitudinal vessel profile category.

There were slightly more medium cross-sectional vessel profiles in the inferior colliculus than in the left cortex in the 24 day old group. These differences were not significant.

4. Neuronal Nuclear Profiles

In the 24 day old left frontal cortex, approximately 74.21 percent of total nuclear profiles counted were neuronal nuclear profiles. This was a higher percentage than found in the corresponding left inferior colliculus (51.74 percent). The average number of neuronal nuclear profiles per unit area in the 24 day old frontal cortex was approximately 578 per mm^2 (N=3) which was higher than the corresponding left inferior colliculus (385 per mm^2). A ratio of neuronal nuclear profiles to one patent small cross-sectional blood vessel per unit area was calculated using the following formula:

$$\frac{\text{Average number of neuronal nuclear profile } mm^2}{\text{Average number of small cross-sect. vessel profiles } mm^2}$$

In the left frontal cortex, in the 24 day old group, this ratio was 1.67:1 and in the corresponding 24 day old left inferior colliculus, this ratio was 0.79:1.

DISCUSSION

The present study has attempted to document, by morphometric analysis, some of the changes occurring in the central region of the inferior colliculus of rats during the first 24 days of postnatal life.

A. Evaluation of Techniques

1. Tissue Shrinkage

It is known that due to tissue preparation methods used, there will be some tissue shrinkage. For example, there is more shrinkage with tissues processed for paraffin sectioning than those tissues that were plastic embedded (epoxy) for electron microscopy (Weibel: 1979). The osmolarity of the fixation solutions has an effect on the cell size and shape. High concentrations of paraformaldehyde and glutaraldehyde in a 0.8 M cacodylate buffer are very hypertonic. Thus lower concentrations of the two fixatives are recommended (for example; 0.5 to 2.0 percent of paraformaldehyde and 1.0 to 3.0 percent of glutaraldehyde) (Glauert: 1974). The osmolarity of solutions has a greater effect on tissues which are "less compact", such as baby rat brains (Hayat: 1981). Therefore, one must be aware of the shrinkage and the possible effects on measurements, especially in the 5 day old brain samples (to be discussed further).

2. Vessel Diameter

It has been established that the maximum diameter of a capillary in the central nervous system of rats was equal to or less than 7.5 microns in cross-section (internal measurement) (Bar: 1980). In this study, the definition of a small vessel profile was slightly larger (10 microns in cross-section, internal diameter) and therefore would include pre- and post-capillary segments. It was noted that the overall average of the patent vessel profile diameters (in the small, cross-sectional vessel profiles) was generally larger in the 5 day old inferior colliculus than was the average generally found in the 24 day old group. In the younger age group, the vessel walls tended to be thicker and not as well defined as was observed in the older age groups. It was reported that the average diameter of capillaries in 8 day old rat brains was 6.75 microns. This decreased to 5.25 microns on day 55 of life (Bar: 1980). Gross et al (1987) reported that the average diameter of capillaries counted in the central nucleus of adult rat inferior colliculus was 4.6 to 4.8 microns. In this current study, the majority of the vessel profiles counted (in the small, cross-sectional profile category) were likely to be equal to or less than 7.5 microns and therefore defined as capillaries.

3. Patent versus Non-patent Blood Vessel Profiles

Only patent vessels were recorded and used in the statistical analysis. Non-patent vessel sprouts were likely present in the younger age groups, especially in the 5 day old group. In order to accurately identify and quantify the presence of vascular sprouts, a marker for endothelial cells, such as alkaline phosphatase, would have to be used (Rowan and Maxwell: 1981b). This was not completed in this study and therefore the discussion will be limited to those patent vessel profiles.

4. Point Counting Method of Estimating Vascular Volume Density

Actual counts of blood vessel profiles provide a more accurate estimation of the number of vessel profiles in a given area, but do not take into account the size of the profile. They do not reflect the amount of the area that is occupied by a vascular component as compared to other components found in the region sampled. One can calculate the relative area occupied by the vessels by measuring the length and diameter of the vessel profiles in a known tissue area. A simpler but well-established manual method to estimate the vascular volume density is the point counting method of Weibel (1979). This method takes vessel profile sizes into account. Since in our coronal sections, the majority of vessel profiles were cross-sections at all ages

studied, there appeared to be no change in overall orientation to the vascular network as a whole. It was therefore appropriate to use point counting as a means to compare different ages for the relative volume fraction of small vessels.

5. Sampling Procedure

The plan was to sample regions at intervals all along the rostro-caudal length of the inferior colliculus, and to restrict the sample to the central part of this structure. As well as spaced serial sectioning, the use of internal landmarks within the colliculus became important for consistency (Plate 1). In general the internal landmarks were convenient to use. However, in the younger age groups, particularly in the 5 and 9 day old group, some of the landmarks in the inferior colliculus were difficult to observe. This posed a difficulty in determining the start point, Level One, that was consistent within the same age group and from age to age. In the older age groups, the landmarks, such as the commissural fibers of the inferior colliculus, were more readily apparent and the starting points (Level One) could be matched from animal to animal and age group to age group. Therefore, in the younger two age groups, Level One may contain a small amount of the central nucleus and more of the external cortex in comparison to the older age groups sampled.

6. Statistical Analysis

The results from using the raw data and the transformed data were presented. For purposes of consistency in the following discussion, references made with respect to significant or non-significant differences will be based on the results of the analysis completed using the transformed data (unless otherwise specified).

B. Changes in the Vascular Pattern in the Rat Inferior Colliculus

The basic vascular framework in the rat central nervous system is established before birth. Additional capillary meshes are added to the framework during the postnatal development of the nervous system by the processes of sprouting and elongation. It was stated by Bar (1983) that the "sprouting process is regarded as an important factor which may determine the effectiveness of the vascular system in the adult animal". The results from this current study indicate an increase in the number of small blood vessels, including capillaries, which was likely due to this process of sprouting or branching to form new blood vessels in the inferior colliculus of the developing rat central nervous system.

The majority of the patent vessel profiles that were counted were found to be small vessel profiles. In the

younger two age groups sampled (the 5 days and 9 days), the small vessel profiles accounted for over 80 percent of the total number counted. In the older two age groups (the 14 days and 24 days), the small vessel profiles accounted for over 90 percent of the total number of vessel profiles counted. In all four age groups sampled most of the small vessel profiles were cross-sections. The percentage of small cross-sectional profiles did increase from the 5 day old group to the 24 day old group. The percentage of small oblique and longitudinal vessel profiles remained stable from 5 days to 24 days.

The medium vessel profile category accounted for less than 20 percent of the total number of vessel profiles counted. Similarly, the majority were cross-sectional vessel profiles. There was, however, a decrease in the percent of medium cross-sectional vessel profiles observed from 5 days to 24 days postnatally.

In all of the four age groups sampled, the largest group of vessel profiles found were small, cross-sectional vessel profiles, largely capillaries. Vessel orientation changed very slightly, primarily between the younger age groups. However, the actual amount of change was only 6 to 7 percent increase in the small, cross-sectional vessel profiles. In the cortex, the random distribution in the orientation of the capillaries has been proved in rats 14 days and older (Eins and Bar: 1978 as cited in Bar: 1980).

There may be slight orientation changes in the younger age groups but the differences do not appear great enough to influence the results.

A possible explanation for the increase in percent of small cross-sectional vessel profiles coupled with a decrease in percent of the medium cross-sectional vessel profiles may be as follows:

The medium size vessels may be the stem vessels that had initially penetrated into the developing brainstem. Within the cortex, newly penetrating stem vessels are not observed after the second week postnatally. With continuing increase in cortical volume, the number of stem vessels per unit area decreased (Bar: 1980). The vascular sprouts develop from the terminal segments of the stem vessels and continue to arise during the second and third week postnatally. Most of the "new" vessels will be classified as capillaries. Thus with decreasing number of newly penetrating vessels (likely medium size vessel profiles) and continuing development of new vessels, the proportion of medium size vessel profiles observed would decrease and the proportion of small size vessel profiles would increase with age.

The vascular volume density, that is, the relative area occupied by a vascular component, increased from 5 days to 24 days in the inferior colliculus. The greatest increase was found in the small cross-sectional vessel profile

category. This would be expected because the largest increase in the number of vessel profiles per unit area was also in the small cross-sectional vessel profile category. There were approximately 70 vessel profiles per unit area in the 5 day old group and this increased to approximately 500 vessel profiles per unit area in the 24 day old group. There were age-related significant differences in both of these measurements in all three age group comparisons. Gross and his co-workers (1987) reported that there were rostro-central-caudal differences with respect to the number of capillaries per unit area in the central nucleus of the adult rat inferior colliculus. They found that the central region of the central nucleus had the highest number of capillary profiles per unit area (540 per mm^2). When one averages the numbers of profiles per unit area from the rostro-central-caudal regions in his study, there were approximately 433 capillaries per unit area, slightly lower than found in this current study for 24 days of age. This may be due to the fact that the current study used a slightly larger internal diameter for defining small vessel profiles. It would appear, therefore, that by 24 days of age, the adult level of vascularity has been reached in the central region of the inferior colliculus.

In the time span from 8 to 20 days postnatally, in the rat cerebral cortex, the endothelial cells proliferate rapidly. It is during this time span that nearly all of the

vascular branches are formed. After 21 days postnatally, there is no detectable further increase in the number of capillaries per unit area (Caley and Maxwell: 1970, Bar: 1980). If a similar pattern of events occurs in the brainstem, then an increase in the numbers of small vessel profiles per unit area would also occur during this time span, and the adult level of vascular density would be reached by 24 days postnatally in the inferior colliculus.

There were also increases in the number of vessel profiles per unit area and in the vascular volume density in the small combined oblique and longitudinal vessel profile category. Within the medium cross-sectional vessel profile category, there was a slight increase in the numbers per unit area and in the vascular volume density between 5 days and 24 days. Most of the age related differences found were not significant. If those medium size vessels are the stem vessels that penetrated into the brainstem and essentially ceased to penetrate during the second week of development, then one would expect to see minimal change in the number of vessel profiles per unit area.

From 5 days to 24 days postnatally, there was an increase in the number per unit area of patent blood vessel profiles containing an endothelial nuclear profile in section. This would be expected because of the large increase in the overall number of vessel profiles per unit area. There was a decrease in the percentage of vessel

profiles containing an endothelial nuclear profile in section. In the 5 day old group, over 55 percent of the vessel profiles observed had an endothelial nuclear profile in section. This decreases to 25 percent in the 24 day old group. This change may reflect the process of elongation where the endothelial cytoplasm is "stretching". In the cortex, during the first week of postnatal development, the average length of the capillaries remains relatively constant. The length begins to increase during the second and third week, ending during the fourth week of development (Bar: 1980). It would appear that a similar time pattern of elongation of the endothelial cells in the central region of the rat inferior colliculus also occurs, with the greatest increase in length found between 14 and 24 days postnatally.

When comparisons were made between the left and right sides in all measurements of vascularity, there were minimal differences found. The data revealed no clear pattern with respect to side differences, and those side differences occasionally observed did not influence the overall trend of increasing vascularity. Since the side differences were inconsistent, it is unlikely that they represent any functional differences, but they could possibly indicate that the left and right sides in any one animal may not mature at identical rates.

C. Changes in the Nuclear Profile Pattern in the Rat Inferior Colliculus.

In the central region of the rat inferior colliculus, there was an overall decrease in the number of nuclear profiles per unit area from 5 days to 24 days postnatally. In the 5 day old sample, there were over 2000 nuclear profiles per unit area. This number decreased to just over 700 nuclear profiles by 24 days of age.

The major change in the nuclear profile pattern occurred in the neuronal nuclear profile and in the glial nuclear profile categories. The percentage of neuronal nuclear profiles (of the total number of nuclear profiles counted - except for endothelial nuclear profiles) decreased from 5 days to 24 days. During the same time, there was a corresponding increase in the percentage of glial nuclear profiles, such that by 24 days of age there was almost a 1:1 ratio of neuronal nuclear profiles to glial nuclear profiles.

It is realized that tissue shrinkage as a result of embedment would make the nuclear profiles more closely packed together - a higher density - especially in the younger age group (5 and 9 days old). But, this tissue shrinkage cannot solely account for the large difference in numerical density that was observed between 5 and 24 days. There was some cell death but it was less than a half percent. It is not known what the exact volume change is

with respect to the inferior colliculus in rats, but the volume is likely increasing with age. Certainly the amount of perikarya around the neuronal nuclear profiles increases, and there is increase in the amount of myelination, all of which contribute to the overall volume change. It is probable that within the inferior colliculus itself, the central nucleus is increasing in size - that is, an increasing amount of the inferior colliculus is occupied by the central nucleus. Also, it is possible that there is continued cell migration both within the central region of the inferior colliculus and away from the central region (to be discussed further). It is likely that all of these factors contribute to the overall decrease in the number of nuclear profiles per unit area from 5 days to 24 days postnatally. A similar decrease in nuclear profile density was observed in the sensory-motor cortex from newborn to 21 days postnatally (Caley and Maxwell: 1970).

In the central region of the inferior colliculus there was a rostro-caudal gradient observed with respect to the neuronal nuclear profiles per unit area. This gradient was more evident in the 5 day old group and to a lesser degree in the 9 day old group. There was no gradient found in the 14 day or 24 day old groups. There was a lower number of neuronal nuclear profiles per unit area in the rostral end of the central region than found in the caudal end.

It has been suggested that there is a dorsocaudal site

of origin where the cells of the inferior colliculus are produced during embryogenesis. It was suggested that the cells were "deployed in an ´outside-in´ pattern" (Altman and Bayer: 1981).

Perhaps there is continued migration of the cells during the first week to 9 days postnatally within the central region of the rat inferior colliculus. The direction of the migration would appear to be from the caudal end towards the rostral end of the central region. It could be argued that in the young rat inferior colliculus the rostral level sampled contained more of the external cortex (which has fewer nuclear profiles) than the central nucleus. It is known that in the adult rat inferior colliculus the central nucleus occupies the medial-caudal two-thirds of the inferior colliculus (Faye-Lund and Osen: 1985). If this were the case, level differences would only be found in the rostral part. In the 5 day old sample, there were level differences throughout the rostro-caudal axis of the central region, more neuronal nuclear profiles in the caudal end. By 9 days, most of the level differences had disappeared but there still were more neuronal nuclear profiles in the caudal region than the rostral region of the central nucleus of the inferior colliculus. The differences between these two regions in the 9 day old group were not as great as the differences found in the 5 day old group. It would therefore appear that neuronal migration continues

during early postnatal development.

There were similar rostro-caudal differences found in the glia nuclear profile category and mainly found in the youngest age group (5 days old). These level differences were not nearly as great as those found in the neuronal nuclear profiles per unit area. The differences found may be related to differences in where gliosis is occurring and migration of those cells.

There were few mitotic and pycnotic figures observed. They were mostly found in the two younger age groups. It is probable that there were more of these figures but they were not observed at the light microscopic level. In the normal developing central nervous system, it has been shown that more neurons are produced than survive to maturity (Silver: 1978). Thus it is probable that the pycnotic figures observed were neurons. Study at the electron microscopic level confirmed that the mitotic figures were glia profiles.

D. Changes in the Vascular Pattern in the Cortex Between Five Days and Twenty-Four Days.

In the cortical region sampled, the left frontal cortex at the level of the optic chiasm, the majority of the vessel profiles observed were small cross-sectional vessel profiles. This was true in both ages sampled. As well, the vascular volume density increased from 5 days to 24 days in both the small cross-sectional and small combined oblique

and longitudinal vessel profile categories. This would be expected because the number of small vessel profiles (both subcategories) per unit area increased from 5 days to 24 days postnatally. Caley and Maxwell (1970) demonstrated a similar increase in vessel profiles per unit area in their study of the postnatal development in the sensory-motor cortex.

E. Comparison Between the Inferior Colliculus and the Cortex at Five Days and Twenty-four Days.

There were no differences found between the left inferior colliculus and left frontal cortex in both age groups sampled with respect to the percentage of vessel profiles that were found to be small cross-sectional vessel profiles. In the 5 day old samples, there were minimal differences found in numbers of vessel profiles per unit area between the central region of the inferior colliculus and the frontal cortex. There were slightly higher numbers of small cross-sectional vessel profiles per unit area in the inferior colliculus than in the cortex. There were no differences with respect to the vascular volume density between the two regions in the 5 day old group.

In the 24 day old group, the differences found between the left inferior colliculus and left frontal cortex were significant. There were higher numbers of small vessel profiles (both in the cross-sectional and combined oblique

and longitudinal vessel profile categories) in the inferior colliculus than in the cortical region sampled. Correspondingly, there was a higher vascular volume density in the inferior colliculus as compared to the cortex. It would appear that there is a richer vascularity in the inferior colliculus than in the cortex in rats.

Significance of Findings

In the central nervous system of rats, there appears to be variation in the density of the capillary network supplying the different functional regions. For example, the capillary density was higher in the rat subfornical organ and the inferior colliculus but lower in the sensori-motor cortex and even lower in the genu of the corpus callosum (Gross et al: 1986). It has been long realized that the vascular density is higher in the gray matter in comparison to the white matter, and, that it appears that the sensory centers of gray matter are more richly supplied then the motor centers of gray matter (Craigie: 1920). It was found in this present study, that such regional differences in vascular density arise during early postnatal capillary growth. At 5 days of age in the rat, the vascular supply to the frontal cortex (at about layers IV and V) and to the inferior colliculus was essentially the same. However, by 24 days of postnatal life, there were significant differences in the "vascular richness" of the inferior

colliculus as compared to the frontal cortex.

In the inferior colliculus, the vascular density was found to be very low in rats less than one week old. However, during the following three weeks of postnatal life in the rat, there was a large increase in the capillary network. This increase was so great, that by 24 days postnatally, the number of capillary profiles per unit area was more than five times the number that was initially found in the 5 day old rat inferior colliculus. This increase in vascularity does begin before the onset of hearing but does not approach adult values until at least two weeks after the initiation of auditory input, along with neuronal growth and maturation. There may be a "factor" or influence that "spurs" on the capillary growth in the inferior colliculus such that this region achieves a greater degree of vascular richness than is observed in most other regions of gray matter. Our data does not indicate when this regional difference is first evident.

It was postulated by Craigie (1920) that differences in capillary density in the various brain regions may be related to differences in the metabolic demands of the different brain regions. In the case of the central nucleus of the inferior colliculus, a positive correlation was found to exist between the high capillary density and the high neuronal activity and metabolic demands as measured by glucose uptake (Gross et al: 1987). However, this type of positive correlation does not necessarily hold true for all

brain regions. In the hypothalamic paraventricular and supraoptic nuclei in rats, there was a high capillary density but "rather low" glucose metabolism demands (Sposito and Gross: 1987). Thus, this "factor" or controlling influence on the development of the capillary network may vary from brain region to brain region, and may involve a combination of extrinsic and intrinsic factors.

CONCLUSIONS

1. The vascular volume density, that is, the relative area occupied by a vascular component, increased from 5 days to 24 days postnatally in both the central region of the inferior colliculus and in the left frontal cortex of the rat. In other words, there was a larger relative area that was occupied by a vascular component in the 24 day old sample than in the 5 day old sample. The greatest amount of increase in both of the brain regions sampled was found to be in the small cross-sectional vessel profile category, (mostly)capillaries). The differences were significant.

2. There was an increase in the number of patent blood vessel profiles per unit area from 5 days to 24 days postnatally in both the central region of the inferior colliculus and in the left frontal cortex. The greatest amount of increase was found in the small cross-sectional vessel profile category. This increase occurred in both of the brain regions sampled. The differences were significant.

3. The number of small cross-sectional vessel profiles per unit area in the 24 day old inferior colliculus was similar to those values reported in the adult rat inferior colliculus. Thus, the adult level of vascularity in the

central region of the inferior colliculus in rats has been achieved by 24 days of age in postnatal development.

4. There was a decrease in the number of nuclear profiles per unit area in the central region of the inferior colliculus from 5 days to 24 days postnatally. This change was more dramatic with respect to the neuronal nuclear profiles than in the glial nuclear profile category. In the 5 day old inferior colliculus, there was a ratio of almost three neuronal nuclear profiles to one glial nuclear profile in a unit area. This decreased to almost a one to one ratio in the 24 day old inferior colliculus.

5. There were rostro-caudal differences found in the central region of the inferior colliculus with respect to the neuronal nuclear profiles per unit area and to a lesser degree, in the glial nuclear profiles per unit area. These differences were notable in the 5 day old inferior colliculus and they were minimal in the 9 day old inferior colliculus. Rostro-caudal differences in neuronal density were absent in the two older age groups sampled.

6. There appears to be a combination of factors including increasing cytoplasmic volume, cell migration, pycnosis and possibly others which influence both the decrease in the overall number of nuclear profiles per unit area and the

rostro-caudal gradient observed.

7. At 5 days of age, in the rat central nervous system, there were minimal differences found with respect to the level of vascularity in the left inferior colliculus and the left frontal cortex. By 24 days postnatally, the left inferior colliculus was more vascularized than the left frontal cortex. Thus, by 24 days postnatally, a regional difference with respect to vascularity (capillary density) is evident in the rat central nervous system.

APPENDIX 1

Test System Trials

Objectives: To choose the appropriate lattice test system for use in the point counting method to determine the vascular volume density of the central region of the inferior colliculus of rats (Weibel: 1979).

Procedure:

Trials with two different double square lattice test systems were completed in two age groups - Group A (5 day old rats) and Group C (14 day old rats),(three rats at 5 days old and two rats at 14 days old).

Test System One ("fine" grid), D16, consisted of sixteen horizontal lines and sixteen vertical lines intersecting each other for a total of 256 points or possible "hits".

Test System Two ("coarse" grid), B36, consisted of twelve horizontal lines and twelve vertical lines intersecting each other for a total of 144 points or "hits". In all trials and subsequent measures using the test system, the broken lines (the "forbidden lines") were respected. If a patent blood vessel profile touched or was crossed by a broken line, then any of the intersections or points over that vessel profile were not counted. If the vessel profile

touched or was crossed by the outer solid line, the point(s) over that blood vessel were counted. In both test systems there were two solid outer lines and two forbidden lines. Therefore, those vessel profiles found in the outer boundaries of the grid had a 50 percent chance of their "hits" being counted and a 50 percent chance of their "hits" not being counted (Plate 3).

To adapt a lattice to our problem we had to consider: (1) the size of each "particle" ie. vessel profile; (2) how close to each other the particles were; (3) the abundance of cross versus longitudinal profiles; (4) print magnification of micrographs; and (5) the distribution of the vessel profile types from rostral to caudal, and age to age (see Weibel: 1979 Chapter Four).

List of Abbreviations Used

L - Left
R - Right
CS - Cross-sectional
Obl - Oblique
Long - Longitudinal

Results:

Comparison of the Vascular Volume Density Between the Two Test Systems

Small Blood Vessel Profile Category at 5 Days and 14 Days

APPENDIX TABLE 1

Test System One

Age and Side	CS Profiles	Obl Profiles	Long Profiles
5 Days (L)	0.44 %	0.17 %	0.18 %
(R)	0.42 %	0.20 %	0.21 %
14 Days (L)	1.43 %	0.50 %	0.46 %
(R)	1.10 %	0.44 %	0.39 %

APPENDIX TABLE 2

Test System Two

Age and Side	CS Profiles	Obl Profiles	Long Profiles
5 days (L)	0.44 %	0.19 %	0.20 %
(R)	0.39 %	0.21 %	0.22 %
14 days (L)	1.31 %	0.54 %	0.49 %
(R)	1.23 %	0.58 %	0.43 %

These tables present the comparison of the vascular volume densities in the small vessel profile category using the two test systems in the two age groups. There were little differences in the results with respect to the average relative area occupied by a vascular component irrespective of which test system - "fine" or "coarse" was used.

APPENDIX TABLE 3

Average Percent of Two or More Hits Occurring Over a Blood Vessel Profile

Age and Side	Test System One	Test System Two
5 Days (L)	0.17 %	0.11 %
(R)	0.17 %	0.14 %
14 Days (L)	0.42 %	0.23 %
(R)	0.40 %	0.32 %

This table shows the average percent of two or more hits occurring of the total number in the two age groups. Thus, the "chance" of a double hit occurring was slightly less using Test System Two than using Test System One.

Average Percent of Vessel Profile Type with Two or More Hits

Right and Left Sides Combined

APPENDIX TABLE 4

Small Vessel Profile Category

Age	Cross-sectional		Oblique		Longitudinal	
	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
5 Days	0.001	0.000	0.033	0.010	0.047	0.050
14 Days	0.003	0.000	0.110	0.050	0.160	0.110

APPENDIX TABLE 5

Medium Vessel Profile Category

Age	Cross-sectional		Oblique		Longitudinal	
	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
5 Days	0.053	0.020	0.030	0.020	0.010	0.020
14 Days	0.083	0.030	0.041	0.030	0.007	0.005

These tables present the average percent of a blood vessel profile type to have two or more hits over it. The vessel profile types that received most of the double (or more) hits using Test System One were small oblique and

longitudinal profiles and medium cross-sectional profiles in both age groups. With Test System Two, small longitudinal vessel profiles had the most double (or more) hits in the two ages sampled.

Conclusions:

It was decided that Test System Two ("coarse" grid) would be appropriate for further use for the following reasons:

1. The values for the vascular volume density were apparently not influenced by the number of intersections or "hits" in the test system used. The grid with the lower number of intersections was adequate to estimate the vascular volume density.

2. The chance of double (or more) hits happening over the same vessel profile was less using Test System Two, especially with respect to the small oblique, longitudinal profiles and the medium size vessel categories. In sections that had more longitudinal profiles, double hits would be more commonly seen. This introduced an error which we reduced by using the coarse grid.

3. Test System Two required less time to utilize than Test System One.

APPENDIX 2

Measurement of Neuronal Nuclear Profile Diameter

Objective: To determine whether the neuronal nuclear profile diameter changed with age in the central region of the inferior colliculus.

Two independent measures were made in the 14 day old sample and one measurement was made in the 5 day old sample.

The two types of measurements were made in the following manner:

A) Area Equivalent Method (Weibel: 1979)

A series of circles with different diameters on an acetate sheet was used. The sheet was placed over the photograph and the neuronal nuclear profile was matched with the appropriate circle. The corresponding circle number was recorded. The circle diameter was calculated into microns using the following formula:

$$\frac{\text{Circle Diameter in mm} \times 1000}{\text{Print Magnification}}$$

This method of measuring neuronal nuclear profile diameter was completed in both age groups sampled. The samples for measuring were taken from the middle level, both right and left sides, two rats per age group.

B) Filar Micrometer Method

Measurements of neuronal nuclear profile diameter using this method was completed in one 14 day old sample. The purpose was to ensure validity of the area equivalent method.

Measurements were made of the neuronal nuclear profiles found in the central region of the inferior colliculus containing one or more visible nucleoli. Two measurements using the Filar Micrometer attached to a Zeiss Light Microscope were made. These were: 1) nuclear profile long diameter and 2) nuclear profile short diameter. These two diameters were averaged and the numerical value converted to average cross-sectional diameter in microns.

Results:

With the Filar Micrometer Method, the average diameter of the neuronal nuclear profiles measured was 9.98 microns, with a range of 7.5 microns to 12.9 microns. In both methods of measurement, the majority of the neuronal nuclear profiles were in the range of 9 to 10 microns in diameter. Thus the usage of the area equivalent diameter to estimate neuronal nuclear profile diameter is valid and the comparison of the estimates in the two ages sampled is also valid.

APPENDIX TABLE 6

Area Equivalent Diameter

Comparison of the Average Percent of Diameter Size between the Two Age Groups Sampled

Neuronal Nuclear Profiles

Age and	Diameter - microns			
Side	4.63	6.48	9.26	11.11
5 Days				
Left	1.05	33.19	57.63	8.13
Right	1.38	34.93	57.78	5.95
14 Days				
Left	0.98	33.34	55.90	9.80
Right	1.43	33.23	53.65	10.95

(at least 200 neurons per side per rat were "measured".)

In both the age groups sampled, the majority of neuronal nuclear profiles were found to be approximately 9.26 um. Secondly there appears to be no change in neuronal

nuclear profile diameter with age.

Conclusions:

1. The use of the area equivalent diameter method is a valid measurement tool for use in measuring neuronal nuclear profile diameter.
2. The neuronal nuclear profile diameter does not appear to change between 5 days and 14 days in the central region of the inferior colliculus in rats.
3. Counts of neuronal nuclear profiles in 5 day old rats can be compared with those in 14 day old rats because the unit of counting does not change in size.

LITERATURE CITED

Altman, J. and Bayer, S.A. 1981. Time of Origin of Neurons of the Rat Inferior Colliculus and the Relations Between Cytogenesis and Tonotopic Order in the Auditory Pathway. Exp. Brain Res., 42: 411-423.

Bar, T. 1980. The Vascular System of the Cerebral Cortex. Adv. Anat. Embryol. Cell Biol., 59: 1-59.

Bar. T. 1983. Patterns of Vascularization in the Developing Cerebral Cortex. In: Development of the Vascular System. Pitman Books, London, p.20-36.

Bar, T. and Wolff, J.R. 1972. The Formation of Capillary Basement Membranes during Internal Vascularization of the Rat´s Cerebral Cortex. Z. Zellforsch., 133: 231-248.

Barr, M.L. and Kiernan, J.A. 1983. The Human Nervous System. Harper and Row, Publishers, Philadelphia.

Betz, A.L. and Goldstein, G.W. 1981. Developmental Changes in Metabolism and Transport Properties of Capillaries Isolated from Rat Brain. J. Physiol., 312: 365-376.

Bosher, S.K. and Warren, R.L. 1971. A Study of the

Electrochemistry and Osmotic Relationships of the Cochlear Fluids in the Neonatal Rat at the Time of the Development of the Endocochlear Potential. J. Physiol., 212: 739-761.

Brightman, M.W. 1977. Morphology of Blood-Brain Interfaces. In: The Ocular and Cerebrospinal Fluids (eds. Bito, L.Z., Davson, H. and Fenstermacher, J.D.), pp. 1-25.

Caley, D.W. and Maxwell, D.S. 1970. Development of the Blood Vessels and Extracellular Spaces during Postnatal Maturation of Rat Cerebral Cortex. J. Comp. Neur., 138: 31-48.

Carpenter, M.B. 1985. Core Text of Neuroanatomy. Williams and Wilkins, Baltimore.

Clopton, B.M. and Winfield, J.A. 1973. Tonotopic Organization in the Inferior Colliculus of the Rat. Brain Res., 56: 355-358.

Clopton, B.M. and Winfield, J.A. 1976. Effect of Early Exposure to Patterned Sound on Unit Activity in Rat Inferior Colliculus. J. Neurophysiol., 39(5): 1081-1089.

Clopton, B.M. and Silverman, M. 1977. Plasticity of Binaural Interaction. II. Critical Period and Changes in

Midline Response. J. Neurophysiol., 40(6): 1275-1280.

Coleman, J.R. and Clerici, W.J. 1987. Sources of Projections to Subdivisions of the Inferior Colliculus in the Rat. J. Comp. Neurol., 262: 215-226.

Conradi, N.G. and Sourander, P. 1980. The Early Internal Vascularization of the Rat Brain. Morphological Studies on Foetuses of Normal and Protein-deprived Mothers. Acta Neuropathol (Berl), 50: 221-226.

Craigie, E.H. 1920. On the Relative Vascularity of Various Parts of the Central Nervous System of the Albino Rat. J. Comp. Neuro., 31: 429-464.

Crowley, D.E. and Hepp-Reymond, M-C. 1966. Development of Cochlear Function in the Ear of the Infant Rat. J. Comp. Physiol. Psychol., 62(3): 427-432.

Dardennes, R., Jarreau, P.H. and Meininger, V. 1984. A Quantitative Golgi Analysis of the Postnatal Maturation of Dendrites in the Central Nucleus of the Inferior Colliculus of the Rat. Devel. Brain Res., 16: 159-169.

David, S. and Nathaniel, E.J.H. 1981. Development of Brain Capillaries in Euthyroid and Hypothyroid Rats. Exp.

Neurol., 73: 243-253.

Faye-Lund, H. and Osen, K.K. 1985. Anatomy of the Inferior Colliculus in Rat. Anat. Embryol., 171: 1-20.

Fenstermacher, J.D., Sposito, N.M., Nornes, S.E., Butler, A.B. and Gross, P.M. 1985. Neurosci. Abstr. 11: 246.3

Fitzgerald, M.J.T. 1985. Neuroanatomy - Basic and Applied. Bailliere Tindall, London.

Geniec, P. and Morest, D.K. 1971. The Neuronal Architecture of the Human Posterior Colliculus. A Study with the Golgi Method. Acta Otolaryng Suppl 295: 1-33.

Glauert, A.M. 1974. Practical Methods in Electron Microscopy. North-Holland Publishing Company, Amsterdam.

Gross, P.M., Sposito, N.M., Pettersen, S.E. and Fenstermacher, J.D. 1986. Differences in Function and Structure of the Capillary Endothelium in Gray Matter, White Matter and a Circumventricular Organ of Rat Brain. Blood Vessels, 23: 261-270.

Gross, P.M., Sposito, N.M., Pettersen, S.E., Panton, D.G.

and Fenstermacher, J.D. 1987. Topography of Capillary Density, Glucose Metabolism, and Microvascular Function Within the Rat Inferior Colliculus. J. Cereb. Blood Flow Metabol., 7: 154-160.

Hamilton, W.J., Boyd, J.D. and Mossman, H.W. 1972. Human Embryology: Prenatal Development of Form and Function. Williams and Wilkins, Cambridge.

Hannah, R.S. and Nathaniel, E.J.H. 1974. The Postnatal Development of Blood Vessels in the Substantia Gelatinosa of Rat Cervical Cord: An Ultrastructural Study. Anat. Rec., 178: 691-710.

Hayat, M.A. 1981. Fixation for Electron Microscopy. Academic Press, New York.

Irvine, D.R.F. 1986. A Review of the Structure and Function of Auditory Brainstem Processing Mechanisms. In: Progress in Sensory Physiology 7 (eds. Autrum, H., Ottoson, D., Perl, E.R., Schmidt, R.F., Shimazu, H. and Willis, W.D.), Springer-Verlag, Berlin Heidelberg.

Jenkins, W.M. and Masteron, R.B. 1982. Sound Localization: Effects of Unilateral Lesions in Central Auditory System. J. Neurophysiol., 47(6): 987-1016.

Ling, E.A., Kaur, C. and Wong, W.C. 1982. Light and Electron Microscopic Demonstration of Non-specific Esterase in Amoeboid Microglial Cells in the Corpus Callosum in Postnatal Rats: a Cytochemical Link to Monocytes. J. Anat., 135: 385-394.

Marin-Padilla, M. 1985. Early Vascularization of the Embryonic Cerebral Cortex: Golgi and Electron Microscopic Studies. J. Comp. Neurol. 241: 237-249.

Masterton, R.B. and Imig, T.J. 1984. Neural Mechanisms for Sound Localization. Ann. Rev. Physiol., 46: 275-287.

Meininger, V., Pol, D. and Derer, P. 1986. The Inferior Colliculus of the Mouse. A Nissl and Golgi Study. Neurosci., 17(4): 1159-1179.

Morest, D.K. and Oliver, D.L. 1984. The Neuronal Architecture of the Inferior Colliculus in the Cat: Defining the Functional Anatomy of the Auditory Midbrain. J. Comp. Neurol., 222: 209-236.

Nousek-Goebl, N.A. and Press, M.F. 1986. Golgi-Electron Microscopic Study of Sprouting Endothelial Cells in the Neonatal Rat Cerebellar Cortex. Devel. Brain Res., 30: 67-73.

Oliver, D.L. and Morest, D.K. 1984. The Central Nucleus of the Inferior Colliculus in the Cat. J. Comp. Neurol., 222: 237-264.

Phelps, C.H. 1972. The Development of Glio-Vascular Relationships in the Rat Spinal Cord. An Electron Microscopic Study. Z. Zellforsch., 128: 555-563.

Repetto-Antoine, M. and Meininger, V. 1982. Histogenesis of the Inferior Colliculus in Rat. Anat. Embryol., 165: 19-37.

Reynolds, E.S. 1963. The Use of Lead Citrate at High pH as an Electron Opaque Stain in Electron Microscopy. J. Cell Biol., 17: 208.

Robertson, P.L., Du Bois, M., Bowman, P.D. and Goldstein, G.W. 1985. Angiogenesis in Developing Rat Brain: an In Vivo and In Vitro Study. Devel. Brain Res. 23: 219-223.

Rowan, R.A. and Maxwell, D.S. 198la. Patterns of Vascular Sprouting in the Postnatal Development of the Cerebral Cortex of the Rat. Amer. J. Anat., 160: 247-255.

Rowan, R.A. and Maxwell, D.S. 1981b. An Ultrastructural Study of Vascular Proliferation and Vascular Alkaline Phosphatase Activity in the Developing Cerebral Cortex of the Rat. Amer. J. Anat., 160: 257-265.

Rubel, E.W. 1984. Ontogeny of Auditory System Function. Ann. Rev. Physiol., 46: 213-229.

Sanes, D.H., Geary, W.A., Wooten, G.F. and Rubel, E.W. 1987. Quantitative Distribution of the Glycine Receptor in the Auditory Brain Stem of the Gerbil. J. Neurosci., 7(11): 3793-3802.

Saunders, N.R. and Møllgard, K. 1984. Development of the Blood-Brain Barrier. J. Devel. Physiol., 6: 45-57.

Schonbach, J., Hu, K.H. and Friede, R.L. 1968. Cellular and Chemical Changes During Myelination: Histologic, Autoradiographic, Histochemical and Biochemical Data on Myelination in the Pyramidal Tract and Corpus Callosum of Rat. J. Comp. Neur., 134: 21-38.

Silver, J. 1978. Handbook of Sensory Physiology Volume IX: Development of Sensory Systems. Springer-Verlag, Berlin, p. 419-436.

Silverman, M.S. and Clopton, B.M. 1977. Plasticity of Binaural Interaction. I. Effect of Early Auditory Deprivation. J. Neurophysiol., 40(6): 1266-1274.

Snell, R.S. 1987. Clinical Neuroanatomy for Medical Students. Little, Brown and Company, Boston.

Sokal, R.R. and Rohlf, F.J. 1969. Biometry. W.H. Freeman and Company, San Francisco.

Sokoloff, L. 1980. The Relationship Between Function and Energy Metabolism: Its Use in the Localization of Its Functional Activity in the Nervous System. Neurosci. Res. Prog. Bull., 19(2): 159-210.

Sokoloff, L., Reivich, M., Kennedy, C., Des Rosiers, M.H., Patalk, C.S., Pettigrew, K.D., Sakurada, O. and Shinohara, M. 1977. J. Neurochem., 28: 897-916.

Sposito, N.M. and Gross, P.M. 1987. Morphometry of Individual Capillary Beds in the Hypothalamo-Neurohypophysial System of Rats. Brain Res., 403: 375-379.

Sposito, N.M., Nornes, S.E., Butler, A.B., Fenstermacher, J.D. and Gross, P.M. 1985. Local Surface Area and Quantitative Fine Structure of Capillaries in White and Grey

Matter and a Circumventricular Organ of Rat Brain. Neurosci. Abstr. 11: 246.4

Stewart, P.A., Hayakawa, E.M. and Hayakawa, K. 1985. Structural Endothelial Changes that Underlie the Maturation of the Blood-Brain Barrier. Neurosci. Abstr., 11: 674 #202.9.

Stoeter, P., Schmidt-Lademann, S. and Voigt, K. 1980. Embryonal and Fetal Development of Capillaries: Microangiographic Investigations. Diag. Imag., 49: 131-140.

Strong, L.H. 1964. The Early Embryonic Pattern of Internal Vascularization of the Mammalian Cerebral Cortex. J. Comp. Neur., 123: 121-138.

Thompson, G.C. and Masterton, R.B. 1978. Brain Stem Auditory Pathways Involved in Reflexive Head Orientation to Sound. J. Neurophysiol., 41(5): 1183-1202.

Wada, T. 1923. Anatomical and Physiological Studies on the Growth of the Inner Ear of the Albino Rat. Amer. Anat. Memoirs, 10: 1-156.

Weibel, E.R. 1979. Stereological Methods. Volume 1. Academic Press, Inc., London.

Wolff, J.R. 1978. Ontogenetic Aspects of Cortical Architecture: Lamination. In: Architectonics of the Cerebral Cortex (ed. Brazier, M.A.B. and Petsche, H.), Raven Press, New York, p. 159-173.

Zilles, K. 1985. The Cortex of the Rat. A Stereotaxic Atlas. Springer-Verlag Berlin Heidelberg, New York.