This dissertation has been
microfilmed exactly as received

66-1797

KING, James Sargent, 1938-
A COMPARATIVE INVESTIGATION OF NEUROGLIA
IN REPRESENTATIVE VERTEBRATES: A SILVER
CARBONATE STUDY.

The Ohio State University, Ph.D., 1965
Anatomy

University Microfilms, Inc., Ann Arbor, Michigan

A COMPARATIVE INVESTIGATION OF NEUROGLIA IN REPRESENTATIVE
VERTEBRATES: A SILVER CARBONATE STUDY

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

James Sargent King, B.S., M.Sc.

The Ohio State University
1965

Approved by

Adviser
Department of Anatomy

ACKNOWLEDGMENTS

The author wishes to thank the following people: Miss Sally O'Neil, for technical assistance; Mr. Vernon Cady, for aid in the preparation of photographic plates; and Mr. Thomas Biggert, for the translation of literature. The author also wishes to acknowledge the valuable counsel and assistance of Dr. Leopold Liss, Department of Pathology; and especially his adviser, Dr. James L. Hall of the Anatomy Department, School of Medicine, The Ohio State University, for help in the preparation of this dissertation.

VITA

November 19, 1938 . . . Born - Painesville, Ohio
1960 B.S., Taylor University, Upland, Indiana
1960-1961 Teacher, Public Schools, Springfield, Ohio
1961-1963 Graduate assistant, Department of Anatomy,
The Ohio State University, Columbus, Ohio
1962 M.Sc., The Ohio State University, Columbus,
Ohio
1963-1964 Assistant Instructor, Department of Anatomy,
The Ohio State University, Columbus, Ohio
1964-1965 Pre-doctoral Fellow, Institute of General
Medical Sciences, Department of Anatomy,
The Ohio State University, Columbus, Ohio
1965 Instructor, Department of Anatomy, The Ohio
State University, Columbus, Ohio

PUBLICATIONS

"The Periperal Sympathetic Nervous System of Necturus Maculosa."
J. Morph., 115: 109-120. July 1964.
"Some Morphological Aspects of Neuroglia in the horse: A Silver
Carbonate Study." Anat. Record, 151: 372. March 1965. (Abstract)

FIELDS OF STUDY

Major Field: Anatomy

Studies in Neuroanatomy. Professor James L. Hall

Studies in Gross Anatomy. Professors Linden F. Edwards and
George R. L. Gaughran

Studies in Comparative Anatomy. Professor W. James Leach

Studies in Histology. Professor G. Adolph Ackerman

Studies in Embryology. Professor John C. Weston

CONTENTS

	Page
ACKNOWLEDGMENTS	ii
VITA	iii
ILLUSTRATIONS	v
INTRODUCTION	1
REVIEW OF THE LITERATURE	3
A. Neuroglia: Comparative Morphology	3
B. Neuroglia: Staining Characteristics and Nomenclature	8
MATERIALS AND METHODS	11
OBSERVATIONS	14
Fish: <u>Dorosoma cepedianum</u>	15
Frog: <u>Rana pipiens</u>	16
Lizard: <u>Sceloporus undulatus</u>	17
Bird: <u>Gallus domesticus</u>	18
Opossum: <u>Didelphis virginianus</u>	20
Horse: <u>Equus caballus</u>	23
Monkey: <u>Macacus mulatta</u>	25
DISCUSSION	29
CONCLUSIONS	36
SUMMARY	38
BIBLIOGRAPHY	40

ILLUSTRATIONS

Figure		Page
1.	Fish. Ependymal cells with elongate processes	45
2.	Fish. Union of ependymal processes into a larger bundle	45
3.	Fish. Sparsity of autonomous neuroglia	45
4.	Fish. Typical bipolar glial cell	45
5.	Frog. Histology of presumptive hippocampal region . .	47
6.	Frog. Tripolar glial cell with secondary branch- ing of processes	47
7.	Frog. Vascular ending in presumptive basal ganglia. .	47
8.	Frog. Blood vessel, neuroglia, neuron relationship. .	47
9.	Frog. Neuron, neuroglia relationship	47
10.	Frog. Glial cell bodies related to blood vessel . . .	47
11.	Lizard. Neuron, neuroglia relationship	49
12.	Lizard. Variation in neuroglia processes	49
13.	Lizard. Presumptive hippocampus, marginal neuroglia	49
14.	Lizard. Neuron, neuroglia relationship	49
15.	Lizard. Vascular ending in basal ganglia	49
16.	Lizard. Ependyma in presumptive hippocampus	49
17.	Bird. Thick and thin processes related to a blood vessel	51
18.	Bird. Neuron, neuroglia relationships	51
19.	Bird. Glial variation in the basal ganglia	51

Figure		Page
20.	Bird. Neuroglia related to neuroglia	51
21.	Bird. Non-ependymal neuroglia in the hippocampus . .	53
22.	Bird. Ependymal cells in the hippocampus	53
23.	Bird. Neuron, neuroglia relations in basal ganglia	53
24.	Bird. Glial variation and glia to glia relations . .	53
25.	Opossum. Oligodendrocytes	55
26.	Opossum. Astrocyte with round cell body in the neocortex	55
27.	Opossum. Astrocyte with round cell body in the hippocampus	55
28.	Opossum. Astrocyte with round cell body in the dentate gyrus	55
29.	Opossum. Stellate astrocyte	55
30.	Opossum. Lamellar astrocyte	55
31.	Opossum. Astrocyte related to dendrites	57
32.	Opossum. Astrocyte relations to dendrites and astrocyte relations to other astrocytes . .	57
33.	Opossum. Neuron, neuroglia relations	57
34.	Opossum. Neuron, neuroglia relations, and astro- cyte related to astrocyte	57
35.	Opossum. Astrocyte in the neocortex	59
36.	Opossum. Neuron, neuroglia relations in the caudate nucleus	59
37.	Opossum. Marginal layer of dentate gyrus	59
38.	Opossum. Marginal layer of neocortex	59
39.	Horse. Variation in astrocyte vascular processes . .	61
40.	Horse. Variation in astrocyte vascular processes . .	61

Figure		Page
41.	Horse. Astrocyte, oligodendrocyte, neuron relations in the caudate nucleus	61
42.	Horse. Astrocyte, oligodendrocyte relations in the precentral gyrus	61
43.	Horse. Neuron, neuroglia relations	61
44.	Horse. Neuron, astrocyte relations and astrocyte to astrocyte relations in the caudate nucleus . .	63
45.	Horse. Neuron, astrocyte relations in the lentiform nucleus	63
46.	Horse. Neuron, astrocyte, blood vessel relationship in the precentral gyrus	63
47.	Horse. Perivascular astrocytes	63
48.	Horse. Hippocampal-dentate gyrus area, neuroglia related to neuroglia	65
49.	Monkey. Astrocytes in marginal cortex	67
50.	Monkey. Precentral gyrus, middle cortical layers, neuron, astrocyte relations	67
51.	Monkey. Astrocytes in deep cortical layers of precentral gyrus	67
52.	Monkey. Astrocytes with thick processes in deep cortical layers	67
53.	Monkey. Linear astrocytes in precentral gyrus	69
54.	Monkey. Astrocyte, blood vessel, neuron relations, astrocyte, astrocyte relations in caudate nucleus	69
55.	Monkey. Astrocyte, astrocyte relations in caudate nucleus	69
56.	Monkey. Globus pallidus with neuron, neuroglia relations	69
57.	Monkey. Hippocampus-dentate gyrus region	71
58.	Monkey. Pyramidal cell layer of hippocampus	71

Figure		Page
59.	Monkey. Astrocyte variation in dentate gyrus	71
60.	Monkey. Glial processes in dentate gyrus	71
61.	Monkey. Vascular relations in precentral gyrus . . .	73
62.	Monkey. Vascular relations in supragranular layers of precentral gyrus	73
63.	Monkey. Vascular process in dentate gyrus	73
64.	Monkey. Astrocytes with round cell bodies in the dentate gyrus	73

INTRODUCTION

Since the first description of neuroglia by Virchow in 1846 (21) a great deal of literature has been written pertaining to its morphology and proposed function. However, there are definite gaps in the anatomical literature pertaining to the neuroglia found in certain species and more specifically, the neuroglia found in specific regions of the vertebrate central nervous system.

The purpose of this investigation is to determine the phylogenetic development of neuroglia in homologous cortical and subcortical forebrain regions of representative vertebrates. The glia are described (1) on the basis of the types of glia found in each animal, (2) by comparing the types of glia found in selected regions in each animal, (3) on the basis of their relations to blood vessels, neurons and other neuroglia. The above histological features of neuroglia are described in the hippocampus-dentate gyrus region, the basal ganglia, and the cerebral cortex (neocortex) or their presumptive areas in the lower animals. These regions were chosen because of their increasing functional importance as one ascends the phylogenetic scale, and because of their early phylogenetic differentiation and retention as distinct morphological areas in higher animals. The animals used represent various classes of vertebrates, including Osteichthyes, Amphibia, Reptilia, Aves, and Mammalia. Representatives of the above

classes were chosen either because of their generalized form or because of the absence of an adequate description of their neuroglial morphology, or both.

The silver carbonate technique of del Rio Hortega (29,30) with Scharenberg's modification (47) was employed because of its specificity for neuroglia and the detailed, precise histological picture that it reveals (9,21). These histological results, however, must be interpreted in the light of recent advances with tissue culture techniques and the electron microscope.

REVIEW OF THE LITERATURE

A. Neuroglia: Morphology

The literature pertaining to the exact species used in this study is sparse; therefore a portion of this section deals with work done on closely related animals. The Golgi method was used by Eurich (15) on Elasmobranch fishes and he reported that the cerebral hemispheres contained only ependymal cells with long processes terminating beneath the pia mater. Horstmann (27) described the neuroglia in the telencephalon of Elasmobranchs in sections stained with Cajal's gold sublimate and also found unipolar ependymal cells that he called "tanycytes." The latter author also described ependymal cells with end feet directly on blood vessels, as well as astrocytes located between the neurons, especially in one particular species. Eurich's paper deals largely with a description of glial elements in the spinal cord of various embryonic vertebrates. But he stated that the cerebrum of the frog (Rana temporaria) contains only ependymal cells with single branched processes. He also reported that the pigeon cerebral cortex has ependymal cells that still reach the periphery and astroblasts which are present in all cortical areas. Muller (36) made an important contribution to the comparative literature, but his descriptions were of the spinal cord of selected vertebrates. In the Teleost spinal cord he described ependymal septa

which are bundles of ependymal processes that are passing from the ependyma to the periphery of the cord. Comparative neuroglia studies on vertebrate spinal cords have also been done by Huber (32) and Pannese (38). Cajal (6) described the neuroglia found in the cerebral hemispheres of the frog. He stated that there are ependymal cells present that send a process to the periphery of the hemisphere and terminate in a cone or cylinder. Achucarro (1) used silver tannin and gold sublimate in his work on the comparative structure of the vascular apparatus in the telencephalon of representative Teleosts, Amphibians, Reptiles and Aves. The fish (Cyprinus auratus) forebrain showed ependymal glia with no vascular insertions, while the frog (Rana esculenta) exemplified a similar morphology, except for thickenings of ependymal processes as they pass over blood vessels. Some processes stop on vessels and form an end foot resembling those found in mammals. In the reptile (Lacerta viridis) the telencephalon has ependymal cells with pial endings that often join and form a triangular end foot. The only non-ependymal neuroglia in the forebrain are found in the presumptive basal ganglia region and septal nucleus (1). The latter author pointed out that Cajal (6) disagreed with this last observation, as Cajal reported only ependymal elements in the telencephalon. Achucarro (1) also reported a great increase in the number of vascular end feet in the septal region, but this was not observed in other presumptive areas of the telencephalon. The histological picture of the corpus striatum of the bird (Fringilla coelebs) was a protoplasmic and fibrous astrocyte framework with only

a few primitive, elongated ependymal cell types (1). Vascular end feet are numerous and many astrocytes form cellular envelopes around the neurones. Cell bodies of astrocytes were also seen to form walls around large blood vessels. He concluded that the vascular attachment of neuroglia precedes autonomous glial elements phylogenetically and suggests the number of glial cells not retaining an ependymal attachment is related to the functional significance of a specific brain region. Penfield (41) stated that in the phylogenetic development of neuroglia, the perivascular foot is substituted for the periependymal foot when blood vessels invade the brain. Neurons also cease to be associated with the ependymal foot processes when the glial cells become autonomous. More recent comparative morphological findings have been reported by Bairati and Maccaguani (4), Contu (8), Bairati and Maccaguani (3), Contu and Maccaguani (10), and Contu (9). These authors described the neuroglia of the telencephalon in a variety of vertebrates stained with the silver carbonate technique of del Rio Hortega. The amphibian forebrain (Bufo vulgaris) and (Rana esculenta) demonstrated a sparse glial network made up almost entirely of ependymal fibers. Satellite cells were lacking around the neurones which was explained by two factors, the lack of neurones and the distance between the ependyma and the pia mater (4). (Lacerta muralis) and (viridis) were used as representative reptiles and the neuroglia forms were predominantly unipolar ependymal cells or bipolar cells with a process directed toward the surface of the brain and the other process directed into the brain substance. No typical stellate forms

of astrocytes were found, but there were some cells that had a few short processes similar to the cell type found in the white matter of mammals. Perineuronal cells were rarely found in either the presumptive hippocampal or basal ganglia regions of the forebrain (8). The chicken (Gallus domesticus) was studied at various ages and it was found that all the types of glia structure were formed by the second month (3). The telencephalon had Bairati's (4) type III classification of glial architecture which is made up of a delicate network of protoplasmic astrocytes, oligodendrocytes and perivascular fibrous astrocytes. These latter cells were related to each other by their processes (3). The neurons have a variable number of satellite cells, but true perineuronal coverings are lacking. Numerous ependymal cell processes and protoplasmic astrocytes with short processes are present in the layers of the pallium (3). The corpus striatum and cerebral cortex of the horse (Equus caballus) were found to have predominantly protoplasmic astrocytes (10). The dominant cell type found in the hippocampus was the fibrous astrocyte which was just the opposite from the rest of the pallium (10). The corpus striatum, cerebral cortex and hippocampus have a glial network of Bairati's type III in the ox (Bos taurus) which was comparable to the horse (10,9). Transitional cells were also seen between protoplasmic astrocytes and fibrous astrocytes and between protoplasmic astrocytes and oligodendrocytes (9). Type III architecture was also present in the cerebral cortex and corpus striatum of the monkey (Macacus rhesus) (9). The predominant cell type found in the hippocampus, molecular and

polymorphous layers of the dentate gyrus of the monkey was the fibrous astrocyte (9). Contu (9) further stated that the primates have a glial morphology similar to man. He also pointed out that the connection between the density of glial structure and size of the nervous system does not exist in mammals. This latter observation has not been confirmed by Brizzee and Jacobs (5), Osorio (37), and Friede (18), who have done work on the glia-neuron index in a number of vertebrates. These investigators stated that the glial density is related to both phylogenetic development and brain size. In addition, the glia-neuron index was found to be related to the length of axones that arise from a particular nucleus (19,37). Hawkins and Olszewski (24) in their study of the glia-neuron index of the whale cortex reported a direct relation between glial density and brain size. The only paper that deals with the neuroglia of the opossum (Didelphis virginianus) was a description of the distribution of oligodendrocytes (7). Cammermeyer (7) also described the distribution of oligodendrocytes in the monkey (Macaca mulatta.) There were a great number of perivascular oligodendrocytes in the deep cortical layers, globus pallidus and a diminished population in the pyramidal cell layer of the hippocampus, putamen and caudate nuclei (7). Throughout the central nervous system of the dog and cat, oligodendrocytes were found to have astrocyte processes ending on them in a blunted or expanded tip (48).

The following is a summary of the observations of Glees (21), Hosokawa and Hajime (31) on the morphology and distribution of neuroglia in various common laboratory animals (cat, dog, rabbit,

monkey) based on silver carbonate, gold sublimate, and Golgi techniques. The superficial layers of the cerebral cortex were found to have small fibrous astrocytes with short processes (21). Numerous perineuronal glial cells were found in the second and third layers of the cortex (21). The protoplasmic astrocyte was the most common cell type found in the cortical layers (21,31). Fibrous astrocytes or transitional forms were found for the most part in the deep layers of the cortex (21,31). Many perineuronal satellites and neuron related astrocytes were described in the basal ganglia (21,31). The globus pallidus due to its relation to white matter was found to contain large fibrous astrocytes (21). Fibrous astrocytes are the predominant cell type found related to neurons and blood vessels in the hippocampus (31). The distribution of neuroglia based on nuclear stains was also discussed by Glees (21). Neuroglia distribution was not found to be essentially different in sections taken from different regions (21,31).

B. Neuroglia: Staining Characteristics and Nomenclature

The histology of neuroglia must be carefully interpreted due to the observations from tissue culture and time lapse photographic techniques. The dynamic activity of the fine protoplasmic membranes and their probable shrinkage upon fixation must be considered when observing sections of metallic impregnations (44,45).

Del Rio Hortega's (28) review and general clarification of the multiple terminology associated with neuroglia suggested that the

following types of neuroglia exist: Protoplasmic astrocytes, fibrous astrocytes, ependymal cells, and four kinds of oligodendrocytes. This classification is based on cell size, number and thickness of processes. There are also intermediate types of astrocytes with characteristics of both kinds (6,9,28). The term oligodendrocyte does not seem to apply to some of the cells identified as oligodendrocytes in the metallic impregnations by De Castro (13). However, in spite of their highly branched appearance, he felt the name should be maintained. Tissue culture studies have revealed that the morphology of the oligodendrocyte is similar in the living and fixed state (13). These cells have also been described as clumping and joining processes in culture (35). Penfield (40) stated that "It is often difficult to say with certainty whether a cell is a large oligodendroglia or a small neuroglia". (astrocyte) (word in brackets is supplied by author). More recent investigations substantiate this latter statement (9,31,12). D'Agata (12) stated that neuroglial forms are so related structurally that it might be better to think of a single glial element. A recent attempt at classification was done by Bairati (2), who reported that the terms protoplasmic and fibrous astrocyte are unacceptable because of the enormous variation in cell size. Also, there is a group of oligodendrocytes which do not correspond structurally to "a cell with few processes." He proposed the following terms: fibrous gliocyte, based on the presence or absence of gliofibrils and other morphological features; oligodendrocyte, either found in white matter or as a perineuronal satellite; epitheloid

gliocyte; transitional gliocytes which have intermediate characteristics between the first four cell types; and ependymal gliocytes. Bairati (2) concluded that the great polymorphism of the gliocytes is not the expression of special functions of the individual cell. The functions of the glia are found essentially in the differentiation that the cytoplasm undergoes and in the complexity of the architecture that the gliocytes form and not in the infinite gradation of their external form (2). Transitional forms similar to oligodendrocytes, the so called "mossy cells", were described by Ramon-Moliner (46) in the cat. Electron microscopic studies also have demonstrated numerous variations and intermediate forms of neuroglia (14,16). Duncan (14) found in the spinal cord of the rat "that intermediate forms are sufficiently numerous to make classification of all cells encountered into astrocytes, oligodendroglia, and microglia an over simplification of a morphologically complex array."

MATERIALS AND METHODS

Seven different adult, vertebrate animals, representing five different classes, and three different orders of Mammals were chosen for this study. Representatives of Osteichthyes, Amphibia, Reptilia, Aves, Mammalia, and the orders Marsupialia, Perissodactyla, and Primates were selected. As a representative of fish, five shad (Dorosoma cepedianum) (LeSueur), were obtained from the Ohio Department of Fisheries. The examples for Amphibia and Reptilia were seven frogs (Rana pipiens), and seven lizards (Sceloporus undulatus), which were secured from the General Biological Supply Co. For Aves, five chickens (Gallus domesticus), were obtained from The Ohio State University, Department of Poultry Science. The above animals were sacrificed by decapitation and their calvaria removed immediately. The whole brain was carefully removed and placed in Cajal's brom formalin (47) within ten minutes after death to insure adequate fixation. As an example of Marsupialia, five opossums (Didelphis virginianus), were obtained from the Carolina Biological Supply Co. For Perissodactyla and Primates, seven horses (Equus caballus), and four monkeys (Macacus mulatta), were secured from the Ohio State University, Department of Veterinary Pathology. The opossums and monkeys were anesthetized with nebutal, decapitated, and then handled in the same manner as above. The horse brains were obtained at autopsy and fixed immediately in Cajal's brom formalin. After an

hour of fixation the larger brains (bird, opossum, horse, monkey) were sectioned transversely every three to four cm. to increase the diffusion of the fixative. The blocks of tissue were then sectioned on a Sartorius-Werke freezing microtome at fifteen micra, after three days of fixation. The entire forebrain of the fish, the frog and the lizard was sectioned and random sections were selected and stained. Fifty to seventy-five sections were cut and stained from each of the selected areas of the bird, opossum, horse and monkey. The cerebral hemispheres of the three lower vertebrates (fish, frog, lizard) were cut in either cross or sagittal sections. These sections remained in Cajal's fixative for periods of time ranging from three weeks to eight months before staining with del Rio Hortega's silver carbonate technique (29, 30). Scharenberg's (47) modification of Hortega's silver method was selected because of the uniform and reliable results obtained with the use of this method. The sections of the selected brain regions, namely, hippocampus-dentate gyrus region, other selected neocortical areas, and the basal ganglia were washed in distilled water, distilled water with twenty drops of ammonium hydroxide for one to three minutes, distilled water for one to two minutes, and then placed in a fifty cc. solution of "weak," "medium," or "strong" silver carbonate, plus twenty drops of pyridine, for forty-five minutes up to four hours, in a 45°C oven. Hyperbromation (heating at 45°C in the fixation medium) before the staining procedure was started resulted in a better impregnation of some regions (29,30). The "medium" or "strong" solution and longer time in the silver bath gave the best results in

the fish, frog, and lizard. The staining was then reinforced with 2 percent ammonical silver nitrate for twenty to thirty seconds, and reduced in 1 percent neutral formalin for one to two minutes. It should be noted that this formalin is made up in five-hundred cc. quantities with the addition of twenty drops of pyridine. The next three steps were washing in distilled water, toning in a .2 percent solution of gold chloride for twenty to thirty minutes, and washing in distilled water. Finally the sections were fixed in a 5 percent solution of sodium thiosulphate, plus a few drops of ammonium hydroxide, washed in distilled water for five minutes, floated on slides, air dried, dipped in xylol, and coverslipped with permount.

Acid cleaned glassware is absolutely necessary for consistent results. Also the glass rod used for transferring sections from dish to dish should be wiped with a piece of gauze between staining dishes to prevent precipitation.

This technique proved to be extremely accurate in demonstrating the processes of neuroglial cells and their relationships to blood vessels, neurons, and to other glia.

The microphotographs were taken on a Zeiss photomicroscope using Adox KB14 black and white film.

OBSERVATIONS

The presence or absence of the following macroscopic brain regions (or their primordium), (1) hippocampus, dentate gyrus, (2) precentral gyrus, (3) globus pallidus, (4) putamen, caudate nuclei, and the use of their phylogenetic nomenclature, (1) archipallium, (2) neopallium, (3) paleostriatum, (4) neostriatum, was based on the work of Crosby, Humphrey, and Lauer (11), Herrick (25), Hoffman (26), Johnson (33), Kappers, Huber, and Crosby (34), and Papez (39). The archipallium or its presumptive area was present in all the animals used in this study. The paleostriatum was considered to be present from fish to man (11). However, functionally the fish forebrain was considered largely an olfactory organ by many authors (33,34,39). Therefore the striatum will not be described as a distinct anatomical region until the Amphibia. Since the representation of the neopallium was so small in the lizard and its specific location not clear in Aves (34), it was not considered as a morphological region until Marsupialia. Analogues of the neostriatum are indicated in Reptilia and Aves, but a distinct caudate nucleus was not observed until Marsupialia. The putamen was not prominent until the primates.

The terms protoplasmic and fibrous astrocytes were not used because of the inability to distinguish gliofibrils in our silver carbonate preparations. The latter cell types could be distinguished

on the basis of cellular shape, and number and thickness of processes, but it was evident that the variations of these classical cell types make this terminology an oversimplification. Astrocytes were described on the basis of the shape of their cell bodies and the number and thickness of processes and simply referred to as astrocytes. The more primitive polar, non-ependymal glial elements (unipolar, bipolar, tripolar) observed in the lower vertebrates were not clearly astrocytes or oligodendrocytes. Therefore, they were generally considered as primitive adaptations of glia cells that had migrated from the ependyma. Absence of perivascular feet, smaller cell bodies, and more delicate expansions were considered as criteria for the identification of oligodendrocytes (31,42). This too was recognized as inadequate terminology because of transitional forms; however, the above morphological characteristics were chosen for our descriptions. These criteria were considered the most accurate in the light of past nomenclature and recent investigations with tissue culture and the electron microscope.

Fish: Dorosoma cepedianum

The neuroglia of this species were for the most part ependymal cells, with their cell bodies lining the first and second ventricles (Fig. 1). Elongate processes were seen to pass into the forebrain which often joined (three to five) to form larger bundles of processes (Fig. 1). These processes (single or bundles) were observed to cross or course in close relationship to both neurones and blood

vessels (Figs. 1, 2). The latter processes could be traced for a considerable distance and terminated just beneath the pia mater in a slight enlargement. The bundles separated, just before their termination, into individual processes. Non-ependymal bipolar and tripolar glial cells were also found and when present were associated with the large multipolar neurons as opposed to the small granule nerve cells (Fig. 4). Figure three demonstrates a typical bipolar glial cell with its cell body closely related to a blood vessel and one of its processes coursing over a neurone. This later relationship was rare as the non-ependymal bipolar elements were not usually seen in physical contact with blood vessels or neurons. However, occasional small, round glial elements with no visible processes were observed in a satellite position to the large neurons. No typical end feet as seen in higher animals were found associated with the blood vessels.

Frog: Rana pipiens

The presumptive hippocampus and striatum of this animal had a predominance of ependymal glial elements (Fig. 5). These cells had single long processes that coursed from their cell bodies to subpial terminations. End feet were observed just beneath the pia mater. The ependymal processes were closely associated with neurons as they passed through the forebrain (Fig. 5). The bundle formation of ependymal processes was not observed in either primitive brain region. However, a glial network was apparent due to the secondary branching

of glial processes (Figs. 5, 6). Non-ependymal glia were present and had round to oval cell bodies with from one to three processes that exemplified various degrees of secondary branching (Figs. 6, 8, 9). When observed in cross section the striatum had more free glial elements in the marginal layer (between the neurons and the pia) in comparison with the hippocampus (Fig. 5). The non-ependymal polar cells were observed with one or more of their processes oriented to the pia or a neuron (Fig. 9). However, the number of neureglia with a process related to a neuron or a glia cell in a satellite position was sparse. The vascular relationships found in this animal were more complex than in the fish. Some cells were found with their cell body directly on a blood vessel and a single process with varying degrees of secondary branching directed away from the vessel (Fig. 8, 10). Cells with a single process wrapped around a blood vessel were also observed (Fig. 7). Occasional vascular end feet from ependymal cells were found, and ependymal processes were also observed to course along the vessel walls. Enlargements or areas of increased thickness were found where the processes were related to a blood vessel.

Lizard: Sceloporus undulatus

Ependymal cells with single, elongated processes were the most common glial cell type found in the presumptive hippocampus and striatum (Fig. 16). In the hippocampus, ependymal processes occasionally bifurcated before their termination and were observed to end in a subpial end foot or in relation to a neuron (Figs. 14, 16).

Vascular end feet or skein type networks were also seen as endings of ependymal cell processes. The processes of the ependymal cells in the primitive neostriatum could not be traced for any great distance. However, in the more ventral paleostriatum subpial terminations were observed. A variety of non-ependymal neuroglia were found ranging from small bipolar elements to large oval or polygonal multipolar cells (Figs. 11, 13, 14, 15). A variation in the thickness and in the secondary branching of processes was observed in some of the larger cells (Fig. 12). The non-ependymal neuroglia were observed with their processes oriented toward neurons and the pia mater in both regions examined (Figs. 11, 13, 14). A few rare glial cells without impregnated processes were found in a satellite position. The vascular relations of the non-ependymal neuroglia were (1) a process with a vascular end foot (Fig. 15), (2) a process without an enlargement related to a vessel, and (3) a cell body lying on a blood vessel with a process directed away from the vessel. The latter vascular related cells were unipolar in most instances. In the paleostriatum an occasional polygonal shaped neuroglia with thick branching processes was found (Fig. 12). On the basis of their morphology these cells could be considered astrocytes. Marginal neuroglia (between the neurons and the pia mater) were observed in both the presumptive striatum and hippocampus (Fig. 13).

Chicken: Gallus domesticus

Unipolar ependymal cells and a number of non-ependymal bipolar and tripolar neuroglia with round cell bodies were observed in the

primitive hippocampus (Figs. 21, 22). The ependymal cells appeared to be more numerous, but as one passes from ventro-medial to dorso-lateral on the dorsal aspect in a transverse section of this region the free elements increased in number (Figs. 21, 22). A complex network of glial processes of ependymal cell origin was observed around the neurons (Fig. 22). Most of their terminations were sub-pial, but as the ependymal processes passed to the pia they were closely associated with blood vessels as well as neurons. The non-ependymal cells were often found in the marginal region and closely associated with neurons (Figs. 21, 22).

Ependymal cells with short processes that could not be traced for any great distance were found along the ventricular border of the paleostriatum. However, there was a greater number of astrocytes and oligodendrocytes present in the striatum as compared to ependymal cells (Figs. 19, 20, 24). The following variety of forms were observed: (1) a typical oligodendrocyte (small, round cell body with two to four processes); (2) transitional forms (cells with morphological characteristics between typical oligodendrocytes and stellate astrocytes); (3) astrocytes (cells with larger, round to slightly polymorphic cell bodies, four to eight processes, and a variation in the degree of secondary branching and thickness of their processes); (4) typical stellate astrocytes (Figs. 19, 20, 24). The cell described above (number 3) was considered to be an astrocyte because of the thickness and number of processes it possessed and because of its relative size as compared to typical oligodendrocytes. There

was also a number of neuroglia that were transitional between the astrocyte with a round cell body and the typical stellate form. The above transitional cells were observed to have a slightly polygonal cell body, and an increase in the thickness and length of their processes (Fig. 23). Astrocytes with round cell bodies and transitional forms toward the typical stellate astrocyte were often found with their processes encompassing neurons and terminating adjacent to the pia mater (Figs. 18, 23). Astrocytes were also present lying directly on the perikaryon of a nerve cell (Fig. 23). Processes of one astrocyte were observed to be in intimate association with the cell body of other astrocytes (Figs. 20, 24). Vascular end feet were found on blood vessels from both the round and stellate forms of astrocytes (Figs. 17, 18). A consistent variation found in the appearance of vascular processes was their thickness (Fig. 17). Some were extremely large, with thick extensions terminating as an end foot, while other terminations were very fine and delicate (Fig. 17). The larger stellate astrocytes were observed related to blood vessels and were also found in the white matter near the neurons of the striatum (Figs. 17, 19, 24). Interfascicular oligodendrocytes were more numerous as opposed to perisomatic oligodendrocytes (Figs. 19, 20, 23, 24).

Opossum: Didelphis virginianus

The ependymal cells of this animal did not have processes that project from the ventricular surface into the brain. A transitional

series of neuroglia was observed from the small round oligodendrocyte to the large stellate astrocyte (Figs. 25, 26, 27, 28, 29, 30). Astrocyte cell bodies varied in appearance from round, to oval, to slightly polygonal, to stellate. The number of processes varied from four to twelve. Astrocytes were observed with fine delicate processes (Fig. 38), heavy thick processes (Figs. 29, 30), and with a combination of thick and thin processes (Figs. 27, 28). The thick processes were often related to blood vessels (Fig. 26), nerve cell processes (Fig. 32), the cell bodies of neurons (Fig. 34), and neuroglia (Fig. 34).

In the three layers of the hippocampus the dominant cell type present was an astrocyte with a round to slightly polygonal cell body (Figs. 31, 32). A few typical satellite oligodendrocytes and astrocytes were found in the pyramidal cell layer (Figs. 31, 32). The latter astrocytic processes were usually associated with the perikaryon of a nerve cell (Fig. 33). Astrocytes were also found related to each other via a close approximation of their cell bodies (Fig. 32). A large number of astrocytes were found with thick processes directly related to the apical dendrites as they radiate from the pyramidal cells (Figs. 31, 32). This latter observation was more characteristic of the pyramidal cell dendrites as one passes from the point of involution of the hippocampus to the region where the pyramidal cell layer blends with the polymorphic layer of the dentate gyrus. Numerous astrocytes with round to slightly polygonal bodies were present in the molecular and polymorphic layers of the dentate gyrus

(Figs. 34, 37). Glial cells were not visualized in the granular cell layer (Fig. 37). However, astrocyte processes were observed closely related to the cell bodies of the latter neurons. The astrocyte cell bodies were located in the polymorphic and molecular layers. Astrocytes of the polymorphic layer were related via their processes to both neurons and other astrocytes (Fig. 34).

The marginal region of the neopallium was sparsely populated with round to polygonal shaped astrocytes as compared with a similar region in the dentate gyrus (Figs. 37, 38). The processes of the above cells were thin and demonstrated little secondary branching (Fig. 38). Relationships were observed between astrocyte processes and blood vessels (Fig. 26), between astrocyte processes and cell bodies of adjacent astrocytes (Fig. 38), and between astrocyte processes and the pia mater (Fig. 38). The cortical layers of neurons appeared to be sparsely populated with neuroglia in comparison to the archipallium (Fig. 35). Satellite oligodendrocytes and oval to polygonal shaped astrocytes were the cell types found in the neocortex. Astrocyte processes generally numbered from two to four with an occasional highly branched cell present (Fig. 35). The above processes were fine and delicate and were often related to neurons (Fig. 35).

Astrocytic elements similar to the cell types found in the cortical layers of the neopallium were seen in both the caudate and lentiform nuclei (Fig. 36). Glial forms were sparse in both these areas of subcortical grey matter, except for a network of glial fibers seen along the ventricular surface of the caudate nucleus.

The thin processes of the astrocytes were often seen contacting neurons (Fig. 36).

Horse: Equus caballus

A transitional series of glial cells similar to that found in the chicken and opossum was observed in the horse. Larger cells (Fig. 40), more polygonal cell bodies (Fig. 46), and more, and thicker processes (Figs. 39, 40, 44, 48) were the principle variations in cellular morphology found in this animal as compared with the opossum.

The pyramidal and molecular layers of the hippocampus showed the following cell types: (1) small, round to slightly polygonal astrocytes (Fig. 43); (2) typical stellate astrocytes; and (3) oligodendrocytes. Small astrocytes with round to polygonal cell bodies were closely related to the large pyramidal neurons (Fig. 43). As contrasted with the opossum, astrocytes were more evenly distributed between the cell body and the apical dendrites of the pyramidal neurons. A variety in the number (four to ten) and thicknesses of the astrocyte processes was observed (Fig. 43). The thicker processes were often seen contacting a neuron or a blood vessel. There appeared to be a general increase in the number of astrocytes in all hippocampal layers as compared with the opossum. The number of satellite oligodendrocytes around the pyramidal cells also appeared to increase in number as compared to the opossum. A dense network of glial processes was observed in both the granular cell and polymorphic layers of the dentate gyrus (Fig. 48). The processes were found closely related to

the granular neurons and had their cell bodies in either the polymorphic layer or molecular layers. The neurons of the polymorphic layer were in intimate contact with both satellite oligodendrocytes and the processes of round to polygonal shaped astrocytes. The processes of the latter cells were observed to have varying degrees of thicknesses. Thick processes were often seen contacting blood vessels, neurons or the processes and cell bodies of other neuroglia (Fig. 48). In the network of glial processes seen in the polymorphic layer, processes of astrocytes appeared to be closely associated with the processes and cell bodies of other astrocytes (Fig. 48). Also oligodendrocyte processes were closely related and at their point of contact with the processes of other oligodendrocytes there appeared to be an increase in the thickness of the processes (Fig. 48). This latter observation was also seen between the processes of small polygonal shaped astrocytes and typical round oligodendrocytes. There were abundant round to polygonal shaped astrocytes with their processes related to the pia mater and to blood vessels in the molecular layer of the dentate gyrus. In the marginal area of the precentral gyrus were found both large stellate astrocytes and small round to polygonal astrocytes (Figs. 40, 47). Processes generally numbered from four to twelve and were often elongate with some degree of secondary branching (Figs. 40, 47). Elongate thin processes were related to blood vessels (Fig. 40), and the cell bodies of astrocytes were also seen lying directly on vessel walls (Fig. 47). Thick processes were demonstrated with typical vascular end feet in other

cortical layers and in the basal ganglia (Fig. 39). In the deep layers of the cortex stellate astrocytes, round to polygonal astrocytes, and satellite oligodendrocytes were found related to neurons (Fig. 46). The thin processes of the astrocytes were oriented toward blood vessels and were seen in contact with the processes and cell bodies of neurons (Fig. 46). Also in the neopallium the processes of astrocytes appeared to end on the cell bodies of oligodendrocytes (Fig. 42).

In the caudate nucleus were found large stellate astrocytes with their thick processes related to blood vessels, neurons, and other astrocytes (Fig. 44). Satellite oligodendrocytes were also observed around the neuron cell bodies (Figs. 44, 45). Astrocytes with oval cell bodies were observed to send their thin processes to neurons (Fig. 41). The cell body or a process of the latter astrocytes was also found closely related to oligodendrocyte cell bodies (Fig. 41). Astrocytes were also present in the paleostriatum, but they were generally the small round to polygonal astrocytes (Fig. 45). The latter cell processes appeared to be in physical contact with neurons as did many satellite oligodendrocytes (Fig. 45). There appeared to be a definite increase in the number of neuroglia in the horse caudate and lentiform nuclei as compared with the opossum striatum.

Monkey: Macacus mulatta

The transitional forms and general cell types of neuroglia described in the opossum and horse were also observed in the monkey. In comparison to the horse and opossum the following general

differences in cellular morphology were observed: (1) an increase in the number, length, thickness and branching of processes (Figs. 55, 60, 64); (2) a more apparent number of vascular relations (Figs. 52, 61); (3) the cell bodies were more pleomorphic (Figs. 49, 53, 64).

Astrocytes with round to polygonal cell bodies were the dominant cell type found in the pyramidal and polymorphic layers of the hippocampus (Fig. 58). These latter cells were often associated with the apical dendrites of the pyramidal cells (Fig. 58), as opposed to the perikaryon. Satellite oligodendrocytes were also associated with the above cell bodies (Fig. 58). Stellate astrocytes were commonly found in the molecular layer of the hippocampus. A dense glial network was found in the polymorphic layer of the dentate (Fig. 60). Typical astrocytes with round cell bodies, as observed in the opossum and horse, were evident (Figs. 59, 60). However, there was an apparent increase in the number of processes per cell and many more polygonal to stellate shaped astrocytes (Figs. 59, 60). The neurons were in intimate contact with the glial processes (Figs. 59, 60). Astrocyte processes were found related to other astrocyte and oligodendrocyte cell bodies (Fig. 59). Relationships between processes of the above glial elements were also noted (Figs. 59, 60). Many of the processes were obviously related by chance. However, others were observed with an apparent increase in thickness at the point where the processes were closely related, as previously noted in the horse. Glial cell bodies were not impregnated in the granular cell layer, but a network of processes from astrocytes in the polymorphic and molecular layers

was observed. Relationships between astrocytes and the pia mater, as well as blood vessels were present in the molecular layer of the dentate gyrus as noted previously in the opossum and the horse.

The precentral gyrus presented a transition of cellular morphology as one passes from the marginal cortex to the deep cortex and white matter. The marginal area demonstrated a variety of cells from stellate astrocytes to oligodendrocytes (Fig. 49). However, the most common cell type present was the round to slightly polygonal shaped astrocyte (Fig. 49). These latter cells were often related to the pia mater or to blood vessels (Fig. 49). A great deal of pleomorphism with many astrocytes related to the perikaryon and processes of the neurons was found in the middle cortical layers (Fig. 50). Satellite oligodendrocytes were apparent in all cortical layers (Fig. 50). The deep cortex was characterized by a general increase in the thickness of the processes of astrocytes (Fig. 52). However, round to oval to stellate astrocytic forms were still observed (Fig. 51). The thicker processes were oriented toward blood vessels and were in contact with neurons (Figs. 51, 52). Typical stellate astrocytes were found in a linear orientation in the white matter adjacent to layer six of the cortex (Fig. 53).

The neostriatum had the following cell types: (1) round to slightly polygonal shaped astrocytes (Figs. 54, 55); (2) oval shaped astrocytes with thin processes (Fig. 54); (3) large stellate astrocytes with thick elongated processes (Fig. 55); (4) oligodendrocytes (Fig. 54). The larger astrocytes were observed with their processes

related to neurons, blood vessels, and to the processes and cell bodies of other astrocytes (Figs. 54, 55). In the caudate nucleus astrocytes were often larger than the neurons they were related to (Fig. 55). In the paleostriatum were found typical small astrocytes with round to oval cell bodies (Fig. 56). The latter cells as well as satellite oligodendrocytes were found related to the larger motor neurons of the globus pallidus (Fig. 56). The processes of the astrocytes were in intimate contact with the neuron cell body and often formed a network around the cell (Fig. 56).

An increase in glia-vascular relationships was apparent in all three regions used in this study. A single process usually thicker than the other processes of the cell was found to end on the vessel walls (Figs. 61, 63, 64). Other processes were observed to terminate by wrapping around the vessel walls (Fig. 52). However, thin delicate processes were also directed toward blood vessels (Fig. 62).

DISCUSSION

This study was undertaken to determine whether a relationship to neuronal complexity and specialization of localized regions of the vertebrate telencephalon would be reflected by neuroglia morphology. In addition, a study of the phylogenetic development of neuroglia morphology might further establish the importance of neuroglia in known areas of complex neuronal activity. Previous authors (15, 6, 1, 4, 8) have reported that ependymal cells were the dominant or only glial element found in the forebrain of the fish, frog and lizard. However, a few primitive, non-ependymal glial cells were found in all the above animals that were used in this study. The autonomous (non-ependymal) neuroglia of the fish (Dorosoma cepedianum), the frog (Rana pipiens), and the lizard (Sceloporus undulatus) generally had round cell bodies with from one to four processes. These cells were found with their processes related to neurons, blood vessels, and the pia mater. The tendency of glial cells to lose their primitive ependymal position and to make secondary connections with blood vessels and neurons was increasingly evident from the fish to the lizard. Vascular attachments in the fish were rare, but a definite increase in the frog and lizard was observed. A true vascular end foot was first observed in the lizard. No major differences in glial morphology of the frog were found in the primitive hippocampus and striatum. A few polygonal,

multipolar neuroglia with branched processes were found in the ventral striatum of the lizard. The term astrocyte was not justified in the three lower vertebrates except for this latter cell. An apparent phylogenetic trend was observed in the following neuroglia-vascular relations: (1) ependymal cell processes were observed passing over vessels (fish); (2) enlargements of ependymal processes were related to vessels (frog); (3) ependymal processes terminated on vessels (frog, lizard); (4) non-ependymal cell bodies were lying directly on blood vessels (frog, lizard); (5) non-ependymal cell processes were wrapped around blood vessels (frog); (6) vascular end feet from non-ependymal cells terminated on vessels (lizard). The above observations were in general agreement with Achucarro (1). The vascular end foot was still apparent as the type of vascular attachment in the chicken, opossum, horse, and monkey. The cell bodies of astrocytes were observed to be directly related to vessel walls from the frog to the horse. A variation in thickness of the vascular processes was noted from the chicken through the monkey. This variation has been found as a morphological difference by other authors (6, 21, 31). However, it should be emphasized as a consistent difference and possible indicator of the functional state of an astrocyte. Enzymatic and ionic differences in astrocytes have strongly implicated this cell in a water-ion transport mechanism between the vascular system and the neuron (17, 20). The morphologic evidence seems to support this theory. From a phylogenetic standpoint the number and thickness of vascular processes increased from fish to monkey. This too supports the above theory, as a higher

degree of neuronal metabolism would require a more efficient transportation system between blood vessel and neuron.

Bairati (2) discussed the functions of glia in terms of cytoplasmic differentiation and concluded that the polymorphism of neuroglia was not an expression of function. Our findings do not substantiate this statement. The anatomy of neuroglia was reflected in the size and shape of their cell bodies, and the number and thickness of their processes. As one ascends the phylogenetic scale from fish to monkey the archipallium, neopallium, and striatum showed a parallel degree of complexity of the above morphological features. The relationship of these cells to neurons, to blood vessels, and to other neuroglia also increased in a similar pattern. The specific functions of neuroglia may be reflected in enzymatic complexity and cytoplasmic differentiation. However, the adaptations of primitive round cells with few processes into larger, polygonal, multipolar cells with many processes suggest a degree of functional difference. This is further confirmed by the intimate relationships found between glial processes, neurons, blood vessels, and other neuroglia. The round to slightly polygonal shaped astrocyte was found from the chicken to the monkey, but this cell was also observed to increase in polarity and in number and thickness of processes from the chicken to the monkey. The cell body described as round may well demonstrate a greater angularity in vivo. This was not apparent, however, because of the possible shrinkage of the fine plasma membranes during fixation as suggested by Pomerat (44, 45). The dynamics of neuroglia must also be considered

when observing static histological sections. Tissue culture studies (44, 45) have indicated that the surface membranes of glial processes are extremely active. They are constantly being extended and retracted, which suggests the functional state of a particular cell. This further indicates that the number of processes a cell displays indicates the functional activity of that particular cell. The morphological changes observed in the unipolar ependymal cell found in the fish, frog, lizard, and chicken also indicate the importance of the adaptation of processes. The following sequence was observed: (1) ependymal subpial terminations (fish); (2) ependymal enlargements as they coursed over blood vessels (frog); (3) ependymal terminations on blood vessels and neurons (lizard). These modifications could be explained by the progressive increase in the thickness of the wall of the forebrain and/or the increased metabolic demands of the neurons.

The approximation of glial cell bodies and/or their processes to neurons was found in all the animals used in this study. Ependymal cell processes were observed in close relationship with neurons in the fish, frog, lizard and the presumptive hippocampus of the chicken. Their processes formed a network around neurons that was evident in the frog, lizard and bird. An increasing number of non-ependymal glia related to neurons was found from the fish through the chicken (just hippocampal area). The latter glial processes were thin and appeared to contact nerve cell bodies. A progressive increase in satellite oligodendrocytes and astrocytes with their processes related to neurons was found in the striatum of the chicken and all the forebrain

regions of the opossum, horse, and monkey. Thin processes were still present in the higher phylogenetic species, but thicker processes were more evident. The greater increase in membrane contacts between glia and neurons presented by thicker processes and satellite oligodendrocytes suggests morphologic evidence of the reported biochemical relations of neurons and neuroglia. Inverse enzymatic changes were reported in neurons and glia following stimulation (23), and some steps in the tricarboxylic cycle appear to be performed more efficiently in glia and others in nerve cells (22). The morphological evidence appears to augment biochemical evidence as one ascends the phylogenetic scale. The more complex the nervous system, the greater the metabolic requirements, and concurrently the greater the number of neuron-glia relations that are observed. The above general trend has been suggested by nuclear counts in glia-index studies (5, 18, 24). These latter studies indicate the number of glia as compared with the number of neurons. This technique yields valuable information; however, metallic impregnations reveal a more complete picture in that processes are stained. Astrocytes were found related to the dendrites of the large pyramidal cells of the hippocampus in the opossum, horse, and monkey. This would place them in a more advantageous position to receive bountons, as has been reported by Scheibel and Scheibel (48). This also places them near the part of the neuron that is receiving a constant bombardment of impulses and whose metabolic demands would be in a constant state of flux.

Close relationships were also found between glial cells and other neuroglia in the chicken (striatum), opossum, horse, and monkey. Astrocyte processes were observed in relation to both astrocyte and oligodendrocyte cell bodies. The cell bodies of astrocytes were approximated to other astrocyte cell bodies. Adjacent astrocyte processes were found in close association in the dense glial network in the polymorphic layer of the dentate gyrus. At the point of approximation there appeared to be an increase in the thickness of opposing processes. Oligodendrocyte processes were also observed in similar relationships in the polymorphic layer of the dentate gyrus. The observation that neuroglia are closely related to other glia via their processes is substantiated by Pomerat's movies of oligodendrocyte cultures. Thickened areas were observed between crossing processes, and processes were observed in physical contact with cell bodies. These relationships were not static, but were apparent as these cells moved in the culture medium. The process-to-process relationship was only observed in the horse and monkey, but the process to cell body association was found in the bird (striatum), opossum, horse, and monkey. The electron microscope has demonstrated a five-layered membrane unit between adjacent oligodendrocyte cell bodies and a similar relationship between astrocyte cell bodies (43). Contacts between cell bodies and processes, and processes with adjacent processes were also reported (43). The blunt tips of astrocyte processes were found to end on oligodendrocytes in the metallic impregnations of Scheibel and Scheibel (48). The assumptions that

astrocytes are the nutritional link between blood vessels and neurons of the central nervous system and that oligodendrocytes have the metabolic machinery to supply nutritive or high energy compounds to the nerve cell are generally accepted. Perhaps the increased thickness between processes of astrocytes and oligodendrocytes or the termination of astrocytic processes on oligodendrocyte cell bodies could be another type of link between the vascular system and the neuron. The astrocyte is usually pictured as directly interposed between the vessel and neuron. The close association of glia cells of the same type suggests a possible nutritional or electrical communication of the neuroglia network of cells and processes.

The observations that relationships between neuroglia, blood vessels and neurons are more complex as one ascends the phylogenetic scale further establishes the functional importance of the glia cell. Also the intimate contacts between the glia cell bodies and processes is suggestive of a degree of complexity not previously mentioned in the literature.

CONCLUSIONS

1. Ependymal cells with elongate processes are the dominant type of neuroglia found in the forebrain of the fish, and in the presumptive hippocampus and striatum of the frog and lizard.
2. Non-ependymal glial cells are present in increasing numbers in similar areas of the forebrain from the fish through the lizard.
3. The bird telencephalon represents a transitional phylogenetic stage from a dominance of ependymal glia to a dominance of non-ependymal glia.
4. The appearance of astrocytes and vascular end feet, similar to those described in mammals, was first noted in the paleostriatum and archipallium of the lizard.
5. Variations in the thicknesses of vascular processes were apparent from the bird to the primate.
6. Astrocytes present a polymorphic transition of cells from small, round to slightly polygonal shaped cell bodies to large stellate forms. These cell types were evident in the chicken, opossum, horse, and monkey.
7. The number, thickness, and degree of secondary branching of astrocyte processes increased from the bird to the primate.
8. An increase in neuron-neuroglia relations in the hippocampus-dentate gyrus, the striatum and the neocortex or their respective

precursors, was found as one ascends the phylogenetic scale from fish to primate.

9. In selected forebrain areas thick glial processes associated with neurons were more apparent from the bird to the primate.

10. In selected forebrain areas the degree of cellular polarity observed in astrocytes increased from the bird through the primate.

11. Astrocyte processes are found related to the cell bodies of other astrocytes and oligodendrocytes in the striatum of the chicken and in the archipallium, neopallium and striatum of the opossum, horse, and monkey.

12. The processes of astrocytes were found in close approximation to each other as were oligodendrocytes. The polymorphic layer of the dentate gyrus of the horse and monkey best exemplified these relationships.

SUMMARY

This study was undertaken to determine the phylogenetic development of neuroglia in specific areas of the vertebrate forebrain. The hippocampus-dentate gyrus region, striatum, and neocortex or homologous, presumptive areas from the following vertebrates were stained with Scharenberg's modification of del Rio Hortega's silver carbonate technique: Fish (Dorosoma cepedianum), Frog (Rana pipiens), Lizard (Sceloporus undulatus), Bird (Gallus domesticus), Opossum (Didelphis virginianus), Horse (Equus caballus), and Monkey (Macacus mullata). Ependymal cells were the dominant glia cells present in the fish, frog, and lizard. Non-ependymal cells were found in increasing numbers from the fish to the lizard. The bird represents a transitional stage between a dominance of ependymal cells and a dominance of non-ependymal cells. Astrocyte variations in morphology are described in the chicken, opossum, horse and monkey. The number, thickness and degree of branching of astrocyte processes are interpreted as morphological adaptations of a basic cell type that suggest a functional importance. The variations in thicknesses of vascular processes is suggested as a functional indicator for astrocytes. Neuron-neuroglia relationships were found to increase as one ascends the phylogenetic scale. The importance of this is discussed. Neuroglia were also found in close

association to other glia in the bird, opossum, horse, and monkey. The number of neuroglia found in relation to blood vessels, neurons and other glia parallels the degree of phylogenetic advancement.

BIBLIOGRAPHY

1. Achucarro, N. De l'Evolution de la Neuroglie et Specialment de ses Relation avec l'Appareil Vasculaire. Trab. Lab. Invest. Biol., 13: 169-212. 1915.
2. Bairati, A. Morfologia e Struttura dei Gliociti. Biol. Latina, 2: 601-659. 1949-50.
3. _____, and Maccaguani, F. Recherche sulla Glioarchitettonica dei Vertebrati. II Ucelli. Monitore Zool. Italiano, 58 Suppl.: 53-55. 1950.
4. _____, and Maccaguani, F. Recherche sulla Glioarchitettonica dei Vertebrati. I Anifibi. Monitore Zool. Italiano, 58 Suppl.: 49-52. 1950.
5. Brizzee, K., and Jacobs, L. The Glia/Neuron Index in the Sub-molecular Layers of the Motor Cortex in the Cat. Anat. Record. 134: 97-105. 1959.
6. Cajal, R. Histologie du Systeme Nerveux de L'Homme et des Vertebres. Vol. 2. Maloine, Paris. 68-71, 79, 106, 504-518, 731-761, 824-846. 1911.
7. Cammermeyer, J. The Distribution of Oligodendrocytes in Cerebral Gray and White Matter of Several Mammals. Am. J. Anat., 107: 107-127. 1960.
8. Contu, P. Recherche sulla Glioarchitettonica dei Reptili (Cheloni, Sauri e Ofidi). Arch. Ital. Anat. e Embriol., 58: 295-320. 1953.
9. _____. Recherche sulla Glioarchitettonica dei Vertebrati: Mammiferi (Cetacei, Edentati, Roditori, Insettivor, Chiroterri, Carnivori, Perissodattili, Artiofatilli, Primati). Arch. Ital. Anat. e Embriol., 52: 101-141. 1954.
10. _____, and Maccaguani, F. Recherche sulla Glioarchitettonica dei Vertebrati. V Ungulati. Monitore Zool. Italiano, 58 Suppl.: 98-100. 1950.
11. Crosby, E., Humphrey, T., and Lauer, E. Correlative Anatomy of the Nervous System. Macmillan, New York. 379, 380, 411, 423, 424. 1962.

12. D'Agata, M. *Richerche sulla Morfolgia e Classificazione dell' Oligodendroglia.* Riv. Neurol., 20: 81-127. 1950.
13. De Castro, F. *Sobre el Compartamiento y Signification de la Oligodendroglia en la Substancia Gris Central y de las Gliocitos en las Ganglias Nerviosos Perifericos.* Arch. de Hist. Nor. y Pathol., 3: 317-343. 1946.
14. Duncan, D. *Light and Electron Microscopic Study of Neuroglia in the Normal Spinal Cord of the Rat.* Anat. Record, 151: 345. 1965.
15. Eurich, F. *Contribution to the Comparative Anatomy of the Neuroglia.* J. Anat. Physiol., 32: 688-708. 1898.
16. Farquhar, M., and Hartmann, J. *Neuroglial Structure and Relationships as Revealed by Electron Microscopy.* J. Neuropath., 16: 18-39. 1957.
17. Friede, R. *The Enzymatic Response of Astrocytes to Various Ions in Vitro.* J. Cell Biol., 20: 5-15. 1964.
18. _____. *The Relationship of Body Size, Nerve Cell Size, Axon Length, and Glial Density in the Cerebellum.* Proc. Nat. Acad. Sci. U.S.A., 49: 187-193. 1963.
19. _____, and Van Houten, W. *Neuronal Extension and Glial Supply. Functional Significance of Glia.* Proc. Nat. Acad. Sci. Wash., 48: 817-821. 1962.
20. Gerschenfeld, H. *et al.* *Function of Astoglia in the Water-Ion Metabolism of the Central Nervous System.* Neurology, 9: 412-425. 1959.
21. Glees, P. *Neuroglia Morphology and Function.* C. C. Thomas, Springfield, 1-37. 1955.
22. Hamberger, A. *Oxidation of Tricarboxylic Acid Cycle Intermediates by Nerve Cell Bodies and Glial Cells.* J. Neurochem., 8: 31-35. 1961.
23. _____, and Hyden, H. *Inverse Enzymatic Changes in Neurons and Glia During Increased Function and Hypoxia.* J. Cell Biol., 16: 521-525. 1963.
24. Hawkins, A., and Olszewski, J. *Glia-Nerve Cell Index for Cortex of the Whale.* Science, 126: 76-77. 1957.

25. Herrick, C. The Morphology of the Forebrain in Amphibia and Reptilia. *J. Comp. Neuro.*, 20: 413-547. 1910.
26. Hoffman, H. The Olfactory Bulb, Accessory Olfactory Bulb and Hemisphere of Some Anurans. *J. Comp. Neuro.*, 317: 120-121. 1963.
27. Horstmann, E. Die Faserghlia des Selachiergehorns. *Zeit. fur Zell. und Mikro. Anat.*, 38: 588-617. 1953-54.
28. Hortege, P. del Rio. La Neuroglia Normal, Conceptos de Angiogliona y Neurogliona. *Arch. de Hist. Nor. y Path.*, 1: 5-71. 1942.
29. _____. El Metodo del Carbonato Argentico: Revision General de sus Tecnicas y Aplicaciones en Histologia Normal y Patologica. *Arch. de Hist. Nor. y Path.*, 1: 165-206, 329-362. 1942.
30. _____. El Metodo del Carbonato Argentico: Revision General de sus Tecnicas y Aplicaciones en Histologia Normal y Patologica. *Arch. de Hist. Nor. y Path.*, 1: 231-244, 577-604. 1943.
31. Hosokawa, H., and Hajime, M. Some Aspects of the Histology of Neuroglia. In: *Morphology of Neuroglia*. Ed. J. Nakai. C.C. Thomas, Springfield, 1-52. 1963.
32. Huber, G. Studies on the Neuroglia in Vertebrates. *Am. J. Anat.*, 1: 45-61. 1903.
33. Johnson, J. The Telencephalon of Ganoids and Teleosts. *J. Comp. Neuro.*, 21: 489-591. 1911.
34. Kappers, A., Huber, C., and Crosby, E. The Comparative Anatomy of the Nervous System Including Man. Vol. III. Hafner, New York. 1256-1455, 1477-1485. 1936.
35. Lumsden, C., and Pomerat, C. Normal Oligodendrocytes in Tissue Culture. *J. Exp. Cell. Res.*, 2: 103-114. 1951.
36. Muller, E. Studien uber Neuroglia. *Arch. Mikro. Anat.*, 60: 11-62. 1900.
37. Osorio, H. Bases Anatomicas de la Unidad Funcional Neurona Glia. *Antioquia Medica*, 14: 275-284. 1964.

38. Pannese, E. Recherches Anatomico-Comparatives et Morphogenetiques sur la Neuroglie de la Moelle Epiniere des Vertebres. Compt. Rend. Assoc. Anat., 44: 561-568. 1958.
39. Papez, J. Comparative Neurology. Hafner, New York. 11, 387-478. 1929.
40. Penfield, W. Oligodendroglia and its Relation to Classical Neuroglia. Brain, 47: 430-452. 1924.
41. _____. Neuroglia and Microglia. The Interstitial Tissue of the Central Nervous System. In: Special Cytology. Vol. II. Ed. E. Cowdry. Hoeber, New York. 1031-1065. 1928.
42. _____. Neuroglia, Normal and Pathological. In: Cytology and Cellular Pathology of the Nervous System. Vol. II. Ed. W. Penfield. Hoeber, New York. 421-472. 1932.
43. Peters, A. Plasma Membrane Contacts in the Central Nervous System. J. Anat., 96: 237-248. 1962.
44. Pomerat, C. Dynamic Neurogliology. Tex. Report Biol. and Med., 10: 885-913. 1952.
45. _____. Functional Concepts Based on Tissue Culture Studies of Neuroglia Cells. In: Biology of Neuroglia. Ed. W. Windle. C.C. Thomas, Springfield. 162-175. 1958.
46. Ramon-Moliner, E. A Study of Neuroglia: The Problem of Transitional Forms. J. Comp. Neuro., 110(2): 157-165. 1958.
47. Scharenberg, K. The Silver Carbonate Technique for the Impregnation of the Astroglia. J. Neuropath. and Exp. Neurol., 19: 622-627. 1960.
48. Scheibel, M., and Scheibel, A. Neurons and Neuroglia Cells as seen with the Light Microscope. In: Biology of Neuroglia. Ed. W. Windle. C.C. Thomas, Springfield. 5-23. 1958.

Fig. 1. Fish. Photomicrograph of ependymal cells and processes in the forebrain. 400X

Arrow indicates the union of three processes into a larger bundle.

Fig. 2. Fish. Photomicrograph demonstrating sparcity of autonomous glial elements. 640X

e ependymal cell processes

n neuron

Fig. 3. Fish. Photomicrograph of bipolar glial cell with its cell body related to a blood vessel and a process related to a neuron. 640X

bv blood vessel

g neuroglia

n neuron

Fig. 4. Fish. Photomicrograph of typical bipolar glial cell in the forebrain. 600X

Arrow indicates bipolar cell

Fig. 5. Frog. Photomicrograph of presumptive hippocampal region demonstrating neurons and ependymal cell processes. 160X

Fig. 6. Frog. Photomicrograph demonstrating tripolar glial cell with secondary branching of processes. 400X

g neuroglia

Fig. 7. Frog. Photomicrograph of presumptive basal ganglia region with neuroglial vascular endings wrapped around a blood vessel. 500X

bv blood vessel

g neuroglia

Fig. 8. Frog. Photomicrograph of a glial cell body on a vessel with a single process directed toward a neuron. 400X

bv blood vessel

g neuroglia

n neuron

Fig. 9. Frog. Photomicrograph of a tripolar glial cell with one process directed toward a neuron. 400X

g neuroglia

n neuron

Fig. 10. Frog. Photomicrograph of glial cell bodies related to a blood vessel. Note secondary branchings of the single process directed away from the vessel. 500X

bv blood vessel

g neuroglia

n neuron

Fig. 11. Lizard. Photomicrograph of presumptive hippocampal region. Note glial cell with fine processes directed toward a neuron. 600X

g neuroglia

n neuron

Fig. 12. Lizard. Photomicrograph of glial cell showing different thicknesses of processes and secondary branchings of main cellular processes. 400X

g neuroglia

Fig. 13. Lizard. Photomicrograph of marginal autonomous neuroglia with processes directed toward free surface of presumptive hippocampal area. 400X

g neuroglia

Fig. 14. Lizard. Photomicrograph of ependymal process and process of a free glial cell related to a neuron. 500X

e ependymal cell process

g neuroglia

n neuron

Fig. 15. Lizard. Photomicrograph of basal ganglia region. Note vascular ending and bipolar glial element. 600X

bv blood vessel

g neuroglia

v vascular ending

Fig. 16. Lizard. Photomicrograph of presumptive hippocampal region demonstrating elongated ependymal cell processes. 200X

e ependymal cell processes

Fig. 17. Bird. Photomicrograph of thick and thin processes related to a blood vessel. 1100X

- bv blood vessel
- p thin processes of an astrocyte
- t thick processes of an astrocyte
- v vascular ending

Fig. 18. Bird. Photomicrograph of the basal ganglia demonstrating neuroglia, neuron relationships. 500X

Arrow indicates astrocyte process related to neuron

- n neuron
- s astrocyte with round cell body

Fig. 19. Bird. Photomicrograph demonstrating glial variation in the basal ganglia. 400X

- a typical stellate astrocyte
- o oligodendrocyte
- s astrocyte with round cell body
- tr transitional cell

Fig. 20. Bird. Photomicrograph of glial variation and neuroglia related to neuroglia. 400X

- a typical stellate astrocyte
- b a glia cell process related to a glia cell body

Fig. 21. Bird. Photomicrograph of non-ependymal neuroglia in the hippocampal region. 500X

Arrows indicate non-ependymal bipolar and tripolar neuroglia

n neuron

Fig. 22. Bird. Photomicrograph of ependymal cells in hippocampal region. Note bipolar glial cell. 400X

Arrow bipolar glial cell

e ependymal cell process

n neuron

Fig. 23. Bird. Photomicrograph to demonstrate the close association of neuroglia and the neurons of the basal region. 700X

d a glia cell process related to a neuron

Fig. 24. Bird. Photomicrograph of basal ganglia demonstrating glia to glia relations and variations of cell types. 500X

a typical stellate astrocyte

b a glia cell process related to a glia cell body

s astrocyte with round cell body

Fig. 25. Opossum. Photomicrograph of oligodendrocytes in subcortical white matter. 400X

o oligodendrocyte

Fig. 26. Opossum. Photomicrograph of an astrocyte with a round cell body related to a blood vessel in the marginal cortex. 500X

bv blood vessel

s astrocyte with round cell body

Fig. 27. Opossum. Photomicrograph of an astrocyte with thick and thin processes in the hippocampus. 600X

s astrocyte with round cell body

Fig. 28. Opossum. Photomicrograph of an astrocyte with thick and thin processes in the dentate gyrus. 800X

s astrocyte with round cell body

Fig. 29. Opossum. Photomicrograph of typical astrocyte in subcortical white matter. 700X

a typical stellate astrocyte

Fig. 30. Opossum. Photomicrograph of lamellar forms of astrocytes in subcortical white matter. 400X

l lamellar astrocyte

Fig. 31. Opossum. Photomicrograph of an astrocyte with its processes oriented toward the dendritic trunk of the pyramidal cells in the hippocampus. 600X

Arrow indicates astrocyte related to axon

dt dendritic trunk

n neuron

s astrocyte with round cell body

Fig. 32. Opossum. Photomicrograph of an astrocyte related to an astrocyte and astrocyte processes related to the processes of neurons in the hippocampus. 500X

Arrows indicate astrocyte processes that are related to axons

c an astrocyte cell body related to another astrocyte cell body

dt dendritic trunk

Fig. 33. Opossum. Photomicrograph of a glial cell in contact with a neuron cell body with a large area of cell membrane contact. 1000X

d a glia cell process related to a neuron

n neuron

Fig. 34. Opossum. Photomicrograph of neuroglia in the dentate gyrus. Note astrocyte related to astrocyte and the enlarged terminal process of an astrocyte found in close approximation to a neuron. 500X

b a glia cell process related to a glia cell body

d a glia cell process related to a neuron

n neuron

Fig. 35. Opossum. Photomicrograph of an astrocyte in neo-cortex. 500X

Arrows indicate processes of astrocyte related to neurons

n neuron

Fig. 36. Opossum. Photomicrograph of glia neuron relationship in the caudate nucleus. 500X

n neuron

s astrocyte with round cell body

Fig. 37. Opossum. Photomicrograph of marginal layer of the dentate gyrus. 200X

s astrocyte with round cell body

Fig. 38. Opossum. Photomicrograph of marginal layer of neo-cortex. 240X

s astrocyte with round cell body

Fig. 39. Horse. Photomicrograph demonstrating thick processes of an astrocyte with an end foot on a blood vessel. 700X

- a typical stellate astrocyte
- bv blood vessel
- t thick processes of an astrocyte

Fig. 40. Horse. Photomicrograph demonstrating thin processes of a large astrocyte directed toward a blood vessel in the precentral gyrus. 480X

- a typical stellate astrocyte
- bv blood vessel
- p thin processes of an astrocyte

Fig. 41. Horse. Photomicrograph of the caudate nucleus demonstrating an astrocyte with its processes directed toward two different neurons and its cell body related to the cell body of an oligodendrocyte. Note ring of oligodendrocytes around the neuron and the approximation of their cell bodies to each other. 700X

- aa astrocyte
- n neuron
- o oligodendrocyte

Fig. 42. Horse. Photomicrograph of precentral gyrus that shows astrocyte processes related to the cell bodies of oligodendrocytes. 480X

Arrow indicates astrocyte process related to oligodendrocyte cell body

- a typical stellate astrocyte

Fig. 43. Horse. Photomicrograph of satellite cells around the cell bodies of pyramidal cells of the hippocampus. 500X

- d a glia cell process related to a neuron

Fig. 44. Horse. Photomicrograph of a caudate nucleus that demonstrates astrocytes related to astrocytes by their processes and the approximation of their cell bodies. Also note astrocyte processes related to neurons and blood vessels. 800X

- a typical stellate astrocyte
- bv blood vessel
- f an astrocyte process related to an astrocyte cell body
- j an astrocyte process related to a neuron
- n neuron

Fig. 45. Horse. Photomicrograph of the lentiform nucleus with small astrocytes related to neurons via fine processes. 400X

- d a glia cell process related to a neuron

Fig. 46. Horse. Photomicrograph of the precentral gyrus with an astrocyte interposed between a blood vessel and a neuron. 400X

- aa astrocyte
- bv blood vessel
- n neuron

Fig. 47. Horse. Photomicrograph of marginal cortex showing perivascular glia cells. 480X

Arrows indicate cell bodies of astrocytes related to the wall of a blood vessel

- bv blood vessel

Fig. 48. Horse. Photomicrograph from hippocampal-dentate gyrus area demonstrating astrocyte processes related to each other, and oligodendrocyte processes related to each other with an apparent increase in surface area at the point of contact. 3500X

- h an oligodendrocyte process related to an oligodendrocyte process with an apparent increase in surface area
- i an astrocyte process related to an astrocyte process
- o oligodendrocyte
- s astrocyte with round cell body

Fig. 49. Monkey. Photomicrograph of marginal cortex to demonstrate variations in the number of processes in astrocytes with round cell bodies. 600X

- a typical stellate astrocyte
- o oligodendrocyte

Fig. 50. Monkey. Photomicrograph of middle cortical layers showing neuroglia related to the cell bodies and processes of neurons. 400X

- aa astrocyte
- n neuron

Fig. 51. Monkey. Photomicrograph of deep cortex demonstrating astrocytes with thicker processes as compared to the more superficial cortical layers. 400X

- aa astrocyte
- t thick processes of an astrocyte

Fig. 52. Monkey. Photomicrograph of deep layers of cortex that shows typical large astrocyte forms with heavy, thick processes. Note wrap around vascular attachment. 640X

- bv blood vessel
- n neuron
- t thick processes of an astrocyte
- v vascular attachment

Fig. 53. Monkey. Photomicrograph of linear arrangement of astrocytes just deep to the gray matter of the precentral gyrus. 400X

Fig. 54. Monkey. Photomicrograph of the caudate nucleus demonstrating astrocyte, blood vessel, and neuron relationship. Note the close association of the processes of astrocytes and the size of the astrocytes compared to the neurons. 400X

aa astrocyte

a typical stellate astrocyte

bv blood vessel

n neuron

Fig. 55. Monkey. Photomicrograph of caudate nucleus demonstrating the processes of astrocytes related to the cell body of an astrocyte and to neurons. Note size of astrocytes as compared to neuron size. 600X

f an astrocyte process related to an astrocyte cell body

j an astrocyte process related to a neuron

n neuron

Fig. 56. Monkey. Photomicrograph of the globus pallidus which has generally larger neurons than the caudate but smaller glial cells. 500X

n neuron

Fig. 57. Monkey. Photomicrograph of area where hippocampus pyramidal cell layer blends with the dentate gyrus. 100X

y pyramidal cell layer of the hippocampus

z granule cell layer of the dentate gyrus

Fig. 58. Monkey. Photomicrograph of pyramidal cell layer of the hippocampus with astrocytes oriented to axons. 160X

y pyramidal cell layer of the hippocampus

Fig. 59. Monkey. Photomicrograph of the dentate gyrus demonstrating astrocyte variation in cell size and number of processes. 500X

s astrocyte with round cell body

j an astrocyte process related to a neuron

Fig. 60. Monkey. Photomicrograph of dentate gyrus demonstrating an increase in glial processes as compared to the opossum and the horse. 400X

Fig. 61. Monkey. Photomicrograph of the white matter of the precentral gyrus demonstrating thick processes of astrocytes directed toward a vessel wall. 640X

- a typical stellate astrocyte
- bv blood vessel
- p thin processes of an astrocyte
- t thick processes of an astrocyte

Fig. 62. Monkey. Photomicrograph of cortical supragranular layers with an astrocyte with fine processes oriented toward a vessel. 500X

- bv blood vessel
- p thin processes of an astrocyte

Fig. 63. Monkey. Photomicrograph of an astrocyte with round cell body in the dentate gyrus with a single process specialized for vascular attachment. 400X

- bv blood vessel
- t thick process of an astrocyte

Fig. 64. Monkey. Photomicrograph of dentate gyrus demonstrating typical astrocytes with round cell bodies. Note specialized process for vascular attachment. 400X

- bv blood vessel
- t thick process of an astrocyte