

THE UNIVERSITY OF MANITOBA

ASTROCYTIC POPULATION DENSITIES IN ACUTELY AND CHRONICALLY
ISOLATED CATS' CEREBRAL CORTEX

by

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ABSTRACT

The purpose of this study was to determine whether acute (12 hr.) and chronic (6-24 months) isolation in the cats' cerebral cortex would have any effect on the astrocytic population density.

Intact, acutely and chronically isolated cortex were ablated from left and right suprasylvian gyri of adult cats, Felis catus (Linnaeus, 1758). Ramon-Moliner's (1957) modification of the Golgi technique was used to impregnate protoplasmic and fibrous astrocytes in all three cortical preparations. Astrocytic population densities were calculated at two millimeter intervals and at the mid-point along the length of intact and isolated cortices.

In all three types of cortical preparations astrocytic population densities were significantly less in upper cortical segments when compared to lower cortical segments.

Pooled astrocytic population density was found to be slightly less in lower cortical segments of acutely isolated slabs when compared by significance tests to pooled lower cortical segments in each of the non-operative cortices.

The astrocytic population density in chronically isolated slabs showed that a significant increase in astrocytic population density had occurred in the upper cortical segments when compared to the density in similar segments in the non-operative chronic homotopes.

The apparent "fall-off" in density of observable astrocytic cells in lower cortical segments of acutely isolated slabs may represent a form of intermediate cellular metabolism and/or physical refractoriness of astrocytes to staining procedures. Localized astrocytosis found to occur in upper cortical segments of chronically isolated cortices is believed to have been a result of an interaction between the following factors: phagocytosis, the requirement for new cells to fill spaces left by degenerating dendrites, the need to transport additional nutrients to the upper cortical half and finally the specific requirements of glucose metabolism in the isolated cortical slab.

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STATEMENT OF THE PROBLEM

Neuronally isolated cerebral cortex in the mammal has often been used as a convenient method of studying the electrical and pharmacological properties of this type of tissue. Histological studies by others have examined the reorganized neuronal population density and neuronal circuitry in the isolated cortical tissue.

The purpose of this histological study was to determine whether acute (12 hours) and chronic (6-24 months) neuronal isolation in the cat's cerebral cortex would have any effect on the astrocytic population density within the isolated tissue. The present study was designed to give a more detailed account of the possible role of astrocytes with respect to (a) evidence of phagocytosis as a result of surgical trauma during the preparation of the isolate, (b) histological evidence of astrocytic neuronal pathways within the isolate and (c) the possible role of astrocytes in forming cellular supportive networks within the isolate.

HISTORICAL REVIEW

1. EMBRYONIC DEVELOPMENT OF NEUROGLIA

Cells Derived from Ectodermal Tissue. During embryonic development of the human brain from the 13.3 mm to 350 mm stage the epithelial lining of the primitive neural tube consists of triangular shaped cells which undergo rapid proliferation and migration (Rudeberg, 1964). Berquist (1932) reported that migration of the cells from the primitive neural tube extended radially and laterally into the adjacent zonal area. Cells termed spongioblasts appear spherical in shape and later differentiate to form astrocytes and oligodendrocytes (His, 1889). There are no reports in the literature of the specific embryonic stage in animal development which can account for the initial morphogenesis of neuroglial cells. The undifferentiated neuroepithelial and germ layer cells were considered by Schaper (1897) to be similar to each other during the process of mitosis.

Cajal (1910) considered spongioblast cells to be differentiated immature neuroglial cells. Neuroglial cells in the chick embryo appear during the thirteenth day; in the human embryo neuroglial cells appear during the third month.

Two cell types differentiate from the primitive neuro-epithelium (Truex and Carpenter, 1969; Ham, 1969).

Spongioblasts, the first cell type, develop from the medulloblast while the neuroblast, the second cell type, arises from the germ layer cells. Spongioblasts invade the neural tube and differentiate to form astrocytes and oligodendrocytes, while neuroblasts differentiate to form nerve cells.

Cells Derived from Mesodermal Tissue. Microcytes, another type of neuroglial cell, are elaborated from mesodermal tissue (Pol. 1965; Ham, 1969). The growth and development of microcytes in brains of mouse, rat and human were described by Field (1953). He found, contrary to the finding of Hortega (1920), that fully developed and delicately branched microcytes were already present in the 11 mm rat, the 13 mm mouse and the 55 mm human embryo. Pruijs (1927) described the origin and the differentiation of microcytes from the ependymal region of foetal and newborn rabbits. Rydberg (1932), using histological techniques, observed transitional cells which were neither spongioblast nor microcyte. These cells were found in the foetus of the human, the rabbit and the cat. Transitional cells which were mitotically active have been observed also in the subependymal layer of the cat, the dog, and the rat (Allen, 1912; Bryan, 1959). Cells in this area were reported to have a high proliferative rate (Lewis, 1968). Kershman (1938) reported that the subependymal layer formed a broad band of neuroblasts and spongioblasts which later differentiate

to form neurons and glial cells respectively. Hortega (1916, 1917), using a combined nuclear and cytoplasmic stain, demonstrated that microcytes were derived entirely from mesodermal tissues. He observed also that during foetal and early postnatal development microcytes were found from the pia-mater and vascular endothelial cells. Lewis (1968), using autoradiographic techniques described the presence of several types of astrocytes and microcytes which were presumably formed from the sub-ependymal layer.

2. MORPHOLOGY

Protoplasmic Astrocytes. This cell type is found in the gray matter of the cerebral cortex (Truex and Carpenter, 1969; Polyak 1965). These cells were observed to have a large nucleus in relation to the overall size of the perikaryon. The nucleus is round or oval in shape with a small amount of chromatin. A nucleolus has not been observed in astrocytes (Schade and Ford, 1965). Light microscopy has shown that the cytoplasm of the perikaryon is scanty and filled with granular materials termed gliosomes (Hyden, 1962). The processes of these cells were noted also to be attached to blood vessels and the pia by means of terminal end-feet or fusa (Teuber, 1960). It was observed that in some instances the perikarya of protoplasmic astrocytes were situated adjacent to neuronal perikarya and were termed neuronal satellites (Truex and Carpenter, 1969). In view of the fact that their processes

extend between nerve fibres of the gray matter and between nerve fibres and blood vessels, protoplasmic astrocytes can be considered to lend valuable support to brain tissues and also to form a medium for nutritional transportation (Schade and Ford, 1965).

Processes of protoplasmic astrocytes constitute a major portion of the neurophile (Singh, 1967). Palay and Peters (1965) observed that these processes were not randomly distributed but were arranged in discernible patterns. The patterns, which vary in different regions, appear to lend evidence in support of a theory that receptive surfaces of neurons are insulated by astrocytic processes from all axonal terminals except those axons which synapse specifically upon the neuron.

Fibrous Astrocyte. Fibrous astrocytes were reported by many authors to be present only in the white matter (Bloom and Fawcett, 1968; Ham, 1969). Perikarya of these cells were observed to lie adjacent to myelinated fibres. These astrocytic processes were noted to run parallel and in between myelinated fibres and to bind the processes to blood vessels. The branches of fibrous astrocytes do not branch extensively and are less tortuous in their pathway than branches found in protoplasmic astrocytes. Ultrafine fibrils, termed astroglial fibrils, were observed in the astrocytic processes by many workers using electron microscopic techniques (Mugnaini and Walberg, 1964) and tissue

cultures (Meller and Haupt, 1967). Histological techniques have demonstrated the presence of ribosomes, mitochondria, Golgi apparatus and a small amount of endoplasmic reticulum in the perinuclear cytoplasm of the astrocytic perikarya (Bourne, 1968).

Special Types of Astrocytes. A special type of protoplasmic astrocytes, termed Bergman's cells or fibres, were observed in the molecular layer of the cerebellum. The perikarya of these cells, situated in the purkinje layer, bear processes which penetrate the plexiform layer as they reach the pia-mater. Palay (1965) observed that in the cerebral cortex an intermediate type of astrocyte exhibited both protoplasmic and fibrous processes. This cell type was observed to lie in the lower layer of the cerebral cortex and was adjacent to the white matter.

Oligodendrocyte. Oligodendrocytes were observed in both gray and white matter (Bloom and Fawcett, 1968; Ham, 1969; Polyak 1965; Nakai, 1963). This neuroglial cell, when compared to the astrocyte, had a smaller perikaryon and a nucleus which was hyperchromatic to basic dyes (Cullin, 1963; Lille, 1954). Processes of oligodendrocytes are not extensively branched, bear few spines and form contacts with blood vessels (Nakai, 1963). Bourne (1968), using glutaraldehyde fixation with electron microscopic techniques, observed the presence of a nuc-

leolus in the nucleus. He also observed densely packed ribosomes, endoplasmic reticulum, mitochondria and Golgi Apparatus in the cytoplasm of the perikarya. Ultrafine fibrils were not reported to be present. In the cerebral cortex the perikarya of oligodendrocytes were often observed to be situated adjacent to neuronal perikarya and were termed perineuronal satellites (Polyak 1965; Ham, 1969).

In the white matter, oligodendrocytes were observed to be arranged in rows between myelinated nerve fibres (Ham, 1969). Maturana (1960), Peters (1960), Palay (1965), and Bourne (1968) have reported that oligodendrocytes are intimately associated with the mechanism of myelination in the CNS. Perikarya of oligodendrocytes were observed to lie adjacent to blood vessels (Ham, 1969). These neuroglia were termed perivascular satellites.

Hortega (1928) described four types of oligodendrocytes and classified them on the basis of their morphology. Type I cells are present in the fore-brain, cerebellum and spinal cord. They lie around blood vessels, neurons and fibre tracts. The perikarya, 15 to 20 μ in diameter, have a spherical or slightly polygonal shape from which numerous fine processes extend towards the nerve fibres.

Type II, termed Cajal cells are 20-40 μ in diameter at the perikaryon, have a polygonal or cuboidal shape and are present in the white matter. Their processes are fewer

and thicker than type I and are intimately associated with nerve fibres.

Type III, termed Paladino cells, are found in both cerebral and cerebellar peduncles, medulla oblongata and spinal cord. Extending from the perikaryon of a Type III cell are both thick and thin processes which project for short distances and lie in contact with nerve fibres.

Type IV, the Schwannoid cell, are either round or bipolar in shape. The perikarya are always in contact with nerve fibres and their processes are short and non-branching. They are found in the peripheral nervous system.

Microcyte. Microcytes were observed to have a small hyperchromatic nucleus surrounded by scanty cytoplasm (Schade and Ford, 1965). Microcytic cells were observed also to have a nucleolus while the cytoplasm contained an abundance of ribosomes and other intra-cellular particles (Bodian, 1964). The processes were noted to be covered with a considerable number of tiny "spines". Microcytes were reported to be distributed throughout the brain and spinal cord. Mori and Leblond (1969), with the aid of electron-microscopy, observed microcytes situated in the expanded portion of a capillary basement membrane. These cells contained a small amount of cytoplasm, a few dense intra-cellular particles and gave rise to a few processes. Evidence was presented also to support the view that these cells could break out of their basement membrane enclosure and enter the surrounding brain tissue.

3. FUNCTIONS OF NEUROGLIA.

There are several accounts in the literature which pertain to the functional importance of neuroglia. It was the considered opinion of Friede and Houten (1962) that this body of knowledge was still insufficient to explain neuroglial function.

In the cerebral cortex neuroglia were observed to lie as satellite cells of both neurons and blood vessels (Mori and Leblond, 1969; Pol yak 1965; Ham, 1969; Bourne, 1968). Neuroglia were considered also to be part of the packing density of nervous tissue and thus contribute significantly to brain volume. Friede (1953) theorized that the retention of brain shape was a function of the leptomeninges.

Cajal (1907), using a gold sublimate method, observed that astrocytic processes formed physical connections with both blood vessels and neurons. Ramon-Moliner (1957) made similar observations using a dichromate method. These contacts were interpreted as having formed a type of diffusion channel for the passage of materials from blood capillaries to neurons. Nakai (1963) and Pol yak (1965) attributed the blood-brain barrier mechanism to these physical contacts. Gray (1959) noted that astrocytes and their processes acted as relatively large water pools during the process of cerebral edema. De Robertis and Gerschenfeld

(1961) reported that astrocytes were also electrolytic pools and may influence the electrical activity of adjacent neurons.

Several accounts in the literature have attempted to link oligodendrocytes with the process of axonal myelination in the central nervous system. Luse (1956) observed that processes from several oligodendrocytes formed circular lamellae around axons in the cerebral cortex. Olmstead (1961), using tissue culture techniques, observed that astrocytic processes and axons were in close contact with each other. He concluded from his studies that astrocytes were linked also with the mechanism of axonal myelination. Walberg (1963), using electronmicroscopy, demonstrated the presence of close contacts between fibrous astrocytes and axons in the white matter. Walberg stated that his observations reinforced the theory of other investigators (Alpers and Haymaker, 1934; Scheibel and Scheibel, 1955; and Olmstead, 1961) that more than one neuroglial cell type contributed to the formation of myelin in the central nervous system.

Klatzo and Miquel (1960) and Gonatas et al (1963) demonstrated that microcytes were phagocytic in function. Schade and Ford (1965) attributed to oligodendrocytes the properties of neurophagia and phagocytosis of fragmented myelin. Lesions induced by freeze dry techniques resulted in an accumulation of extracellular spaces and slight swelling of neuroglial cells (Gerschenfeld et al, 1959;

Raimondi et al, 1962; Klatzo et al, 1962). It has long been established that neuroglial cells and their processes form a major part of interneuronal spaces in the brain (Hyden, 1962). It is therefore hypothesized in this thesis that the accumulation of interneuronal fluid should affect the size and fluid content of neuroglial cells. Bankay (1965), using electronmicroscopy, made a careful study of clinically and experimentally induced brain edema. He was able to demonstrate that under such conditions neuroglial cells became swollen with no increase in interstitial volume during the induced swelling. Koelle (1962) reported that glial cells contain a large amount of cholinesterase for which no specific function was reported. Hertz (1965) suggested that glial cells act as carriers of potassium ions from one neuron to another. He therefore concluded that neuroglial cells may act as a mediator of a nerve stimulus between two neurons.

Quantitative Studies of Neuroglial Cells in the Central Nervous System.

During conditions of tissue culture glial cells were observed to proliferate and to exhibit amoeboid movement (Lumsden and Pomerat, 1951; Pomerat and Costero, 1956; Hild, 1957; Peterson and Murray, 1960). Altman (1969) reported that glial cells reproduce by amitotic division. Galambos (1961) reported that the population of neuroglial cells in the brain is approximately ten times greater than the estimated number of neurons. Blinkov and Glezer (1968)

reported that one mm³ of brain tissue was estimated to contain 80,000 to 100,000 neuroglial cells. They reported also that the greatest percentage of neuroglial cells consisted of astrocytes.

The importance of quantitative studies on neuronal and glial populations has several applications. The current literature has shown that neuroglial cells are associated with the following brain conditions: histopathology brain trauma, toxicity, infectious trauma, edema and metabolic disturbances which are the result of deficient blood supply.

Earlier studies on the analyses of histo-pathological changes in the CNS have required an examination of the changes associated with cell shape and cell population. In the central nervous system these studies are directed also towards changes in cell densities of neuroglial and neuronal components.

Nissl (1898) and Von Bonin (1948) compared brain size and cell density in several different animals in the phylogenetic scale of mammals. They reported a lower density of neurons in the cerebral cortex of large brains when compared to the density of small brains. These findings led them to hypothesize that the size of interneuronal spaces would always be greater in animals with a large cerebral cortex. It could therefore be hypothesized that animals with large brains would have a greater population of glial cells than animals with smaller brains.

Friede (1954) studied the glial index, i.e., the number of glial cells per nerve cell, in the telencephalic cortex in several species of animals. He reported that the glial index increased in the phylogenetic scale from frog to mammal. These conclusions were later contradicted by Hawkins and Olszewski (1957). They compared the brains from two adult blue whales, Balaenoptera physalis, with human brains. They reported that the glial index was related to brain size rather than phylogenetic hierarchy. They found that the glial index in the whale brain was 4.54 while in the human it was 1.78. Milman (1936) and Arutyunova (1938) reported that in the superior frontal gyrus of the human brain the glial-index ranges from 0.2 to 2.1. Brizze and Jacobs (1959) reported that the mean glial index for all gyri in the cats' cerebral cortex is 1.50. Blinkov and Glezer (1968), using results previously reported by Ramon-Moliner (1961), calculated the mean glial-index in the cat as 2.0. Hawkins and Olszewski (1957) calculated the mean glial index in the human brain as 1.78. Brizze and Jacobs (1959) showed that the glial index in the cortex of kittens 60 days old with a brain weight of 13.20 grams was 1.00. In adult cats with a brain weight of 18.0 grams the glial index is 1.50. Milman (1936) found that the glial index in the cerebral cortex of newborn guinea pigs was 0.08-0.22 while in the adult of the same species it was 0.70-1.50. Friede (1963) reported that the glial index of large neurons, such as Betz cells, was

higher when compared to pyramidal cells in layer I to V. Reiffenstein (1964), using light microscopy techniques, reported that in chronically isolated cerebral cortex there were significant increases in astrocytic and microcytic populations. Using a Golgi stain, Reiffenstein estimated that in chronic slabs the astrocyte population was four times greater when compared with acutely isolated cortex. He reported also that the Golgi stain showed a population loss in areas around the outer lesions.

Chow and Dewson (1966) reported a decrease in neuronal population in the lateral geniculate body of rabbits during periods of retrograde degeneration. They reported also an increase in the glial population in the early stages following surgical ablation.

METHODS AND MATERIALS

This histological study used intact, acutely and chronically isolated cortex from the left and right supra sylvian gyri of adult cats, Felis domesticus (Linnaeus, 1758). A total of 13 cats was used in this study. The cortical preparations used were as follows: (a) four intact left gyri and their homotopes; (b) three chronically isolated slabs and their homotopes and (c) three acutely isolated slabs and their similarly prepared homotopes. Paraffin sections were prepared also from formalin fixed tissues of intact and homotope cortex taken from one cat. Several experimental stain techniques were conducted on four intact suprasylvian gyri taken from two cats.

1. SURGICAL PROCEDURES

Intact Cerebral Cortex. Four cats were used for this part of the study. The cats were anaesthetized by sodium pentobarbital (Nembutal) administered intraperitoneally. The anaesthetizing dosage was previously determined by Barnes and Eltherington (1965) to be 35 mg/kg weight of cat. The lethal dose used was between 80 and 100 mg/kg. After loss of consciousness the animal was placed on its ventral aspect on an operating table and surgical procedures for craniectomy were followed by methods previously described by Weisman (1969).

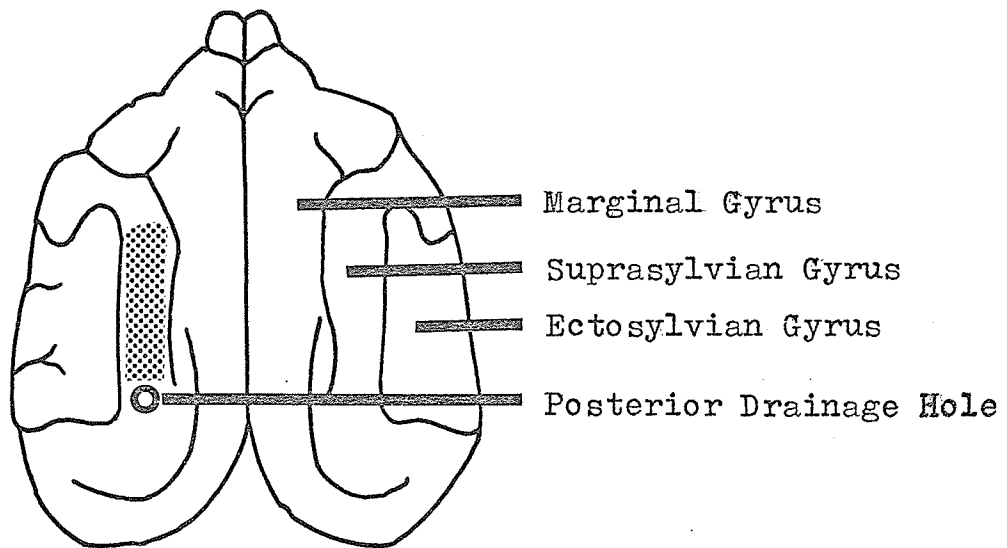


Figure 1. Dorsal Aspect of Mature Cat's Cerebral Hemispheres to Show Area of Study.

The head was fitted in a Tschemakholder which kept it in a semi-rigid position. A mid-line dorsal incision was made on the scalp and the integument was separated from the underlying facial and temporal muscles. These muscles were excised in order to expose posteriorly the calvarium as far back as the lambroidal ridge and laterally on both sides to the zygomatic arch. An oval area 2 cm by 1.5 cm of calvarium situated above the suprasylvian gyrus was circumscribed by an electrically powered dental drill fitted with a No. 8 vanadium steel dental burr. The area was maintained moist by saline kept at 37°C. A bone rongeur was used to enlarge the exposed area. Great care was taken so as to prevent any destruction of the dura mater. Needle nose iris scissors (Irex) were used to remove the dura mater, leaving the pia-mater in its normal state. A No. 11 scalpel blade fitted on a number 3 blade handle was used to (Fig. I.) circumscribe and to cut a block of cortical tissue measuring 25 mm x 12 mm. The cuts were made in the adjacent marginal and ectosylvian gyri. A number 22 scalpel blade fitted on a number 4 blade handle was used to make a horizontal undercut 8 mm deep on the incised cortex. This cut facilitated the separation of the gyrus from the underlying tissues. Upon removal of the gyrus the posterior end was marked for future reference. The tissue block was kept moist with saline kept at 37°C. The excised left and right gyri were placed in separate dark bottles containing the fixative. The animal was then sacrificed by an overdose of Nembutal.

Acutely Isolated Slabs. Acutely isolated slabs were prepared in both left and right gyri by techniques described by Weisman (1969). Acutely isolated slabs were intermittently electrically stimulated over a period of 12 hours. The stimulating current applied was between 100 microamps and 500 microamps, 1 pulse every 5 seconds with a pulse duration of 100 microseconds. Following the conclusion of the electrophysiological experiments the gyri were excised using techniques previously described for the intact cortex. The gyri were rinsed quickly with saline and placed in separately marked dark brown bottles containing the fixative.

Chronically Isolated Slabs. Chronically isolated slabs were prepared as described by Pinsky (1961) and Weisman (1969). Chronicity extended over a period between 15 to 36 months. Neuronal isolation for both chronic and acute slabs was prepared by similar techniques which used two side cuts and one single undercut. The left gyrus was neuronally isolated while the homotope gyrus remained intact. Re-exposure and removal of the left gyrus involved the teasing away of newly formed fibrous adhesions and regenerated bone. The pia mater was not disturbed nor injured. Both gyri were excised by techniques described above for the intact gyrus. Tissue blocks were kept moist with warmed saline kept at 37° C and were placed immediately in separate dark bottles containing the fixative.

2. HISTOLOGICAL TECHNIQUES

Several staining and impregnation techniques have been used successfully to observe each of the different types of neuroglial cells (Cajal 1916; Hortega 1917; Penfield 1924, 1930; Ramon-Moliner 1957, 1958). No single technique is known to impregnate successfully the three-types of neuroglial cells. The nuclei of the different neuroglial cells can be distinguished from each other only by using nucleophilic stains such as cresyl violet, thionin, methylene violet and Haematoxylin-Eosin. The processes and soma of the different neuroglial cells can be distinguished from each other only by a metallic impregnation technique. Most of these metallic impregnation techniques use silver nitrate, which is based on the original Golgi (1873, 1885) method. Several modifications of Golgi's method have been reported (Sala 1893; Strong 1895; Lachi 1895; Fish 1895; Hortega 1928; Cajal 1933; Davenport and Combs 1954). Protoplasmic and fibrous astrocytes have been observed by the use of Cajal's Gold sublimate (1916). Ramon-Moliner's (1957) modification of the Golgi technique was found to be the most suitable for the demonstration of both types of astrocytes in all three cortical preparations. i.e. intact, acutely and chronically isolated cortex.

3. FIXATION PROCEDURES

The following mixture of solutions was used for fixation:

- 40 ml of 6% aqueous potassium dichromate
- 20 ml of 5% aqueous potassium chlorate
- 30 ml of 20% aqueous chloral hydrate and
- 10 ml of 38% formaldehyde

Stock solutions of each of the above solutions were prepared and kept in separate bottles. Solutions were mixed just prior to use. Brain tissues were cut in pieces measuring 4 mm x 6 mm in order to facilitate easy and total penetration.

Extreme care was taken to maintain the anterior to posterior orientation of the gyrus. This was accomplished by making a small notch on the posterior end of each block of tissue. The tissue blocks were transferred to separate glass jars containing a volume of fixative which was 20 times that of the individual tissue block. Optimal fixation time was found to be 3 days. Solutions were changed each day and followed by gentle agitation. Tissue blocks were then separately transferred to a 3% potassium dichromate solution. The volume of the chromate solution was 20 times that of the tissue blocks. The solution was changed twice daily over a period of 3 days.

4. METALLIC IMPREGNATION

A 1% w/v silver nitrate solution, was used for the impregnation of astrocytes. The tissues, prior to their immersion in the silver nitrate solution, were quickly washed in distilled water in order to prevent the formation of a

precipitate reaction between potassium dichromate and silver nitrate. The volume of the silver nitrate solution was 20 times that of the tissue blocks. The tissues were immersed in silver nitrate solution for 3 days with periodic agitation.

5. SECTIONING AND MOUNTING

Serial frozen sections 40 microns in thickness were cut on a horizontal clinical microtome, Sartorius Werk Model 27 No. 20690. The tissue block was positioned on a metal stage connected to a Histo-Freeze, Model No. 3905-1. The Histo-Freeze was electrically powered and was capable of either lowering or raising the temperature of the stage. Sections were cut with a Type B microtome knife. To cut sections of uniform thickness, the knife holder of the microtome was racked only once to its maximum length. It was noticed that any attempt to cut frozen sections of a higher thickness resulted in sections of varying thicknesses.

Serial sections were taken at two millimeter intervals and at the midsection along the length of the gyri. The frozen sections were stored in absolute alcohol for a minimum of two hours. The sections were then transferred to a solution of Xylol for only one minute. Sections were then mounted on pre-cleaned microscopic slides measuring 25 mm x 75 mm. Permount (Fisher Brand SO-P-15) was used as the mounting fluid. Corning cover slip No. 1 thickness was used. Slides were left to dry at room temperature.

6. PARAFFIN PREPARATION

Formalin fixed material was processed on a Shandon Elliott model No. 1742, automatic tissue processor. The tissues were embedded in paraffin, Fisher Brand No. T-299-12-647E. Serial sections, 10 microns thick, were cut at 2 millimeter intervals on a rotary microtome, Jung, model No. 27445. Sections were stained with 1% Cresyl violet (Cullin 1963) and examined under light microscopy at X500 and at X1250 magnification. This technique stained all the nuclei and was used to calculate the percentage of astrocytes impregnation.

7. ANALYTICAL METHODS

The purpose of this study was to determine whether acute and chronic neuronal isolation in the cat's cerebral cortex would have any effect on the astrocytic population density. All microscopic observations on intact and isolated cortical slabs were made by means of light microscopy. A Cooke-Troughton and Sims microscope, model No. 72 1602, was used. It was fitted with Huygenian oculars and flourite objectives X5, X10, X40.

The microscope was fitted also with a mechanical stage and a built in illumination source. The size of protoplasmic astrocytes was found to vary between 25 and 50 microns in diameter. It was found that reliable cell counts could be made at X50 magnification. In the event of clumping of astrocytic cells a higher magnification of X125 was used to facilitate the cell count.

The micrometer ocular inserts, one sub-divided into 100 squares per square millimeter and the other marked with a linear scale of 100 sub-divisions to the millimeter were superimposed upon each other in the same eye-piece. The inserts were calibrated by a stage micrometer sub-divided into 100 sub-divisions. A similar technique of ocular inserts was previously used to enumerate neuron population density (Weisman et al, 1967).

Intact Cortex. An area of intact cortex, 3000 microns in width was demarcated on coronal sections. This area corresponded to similar areas in acutely and chronically isolated slabs. Coronal sections were sub-divided into upper and lower cortical halves. The upper half was sub-divided into four equal cortical segments, two segments lying on either side of the mid-point of the gyrus width (Fig. 2). The lower cortical half was divided into two cortical segments of equal width.

Acutely Isolated Cortex. Coronal sections of acutely isolated cortex were sub-divided into six cortical segments by methods similarly described for intact cortex. The sub-divisions were made within the acute slab as shown in Fig. 3.

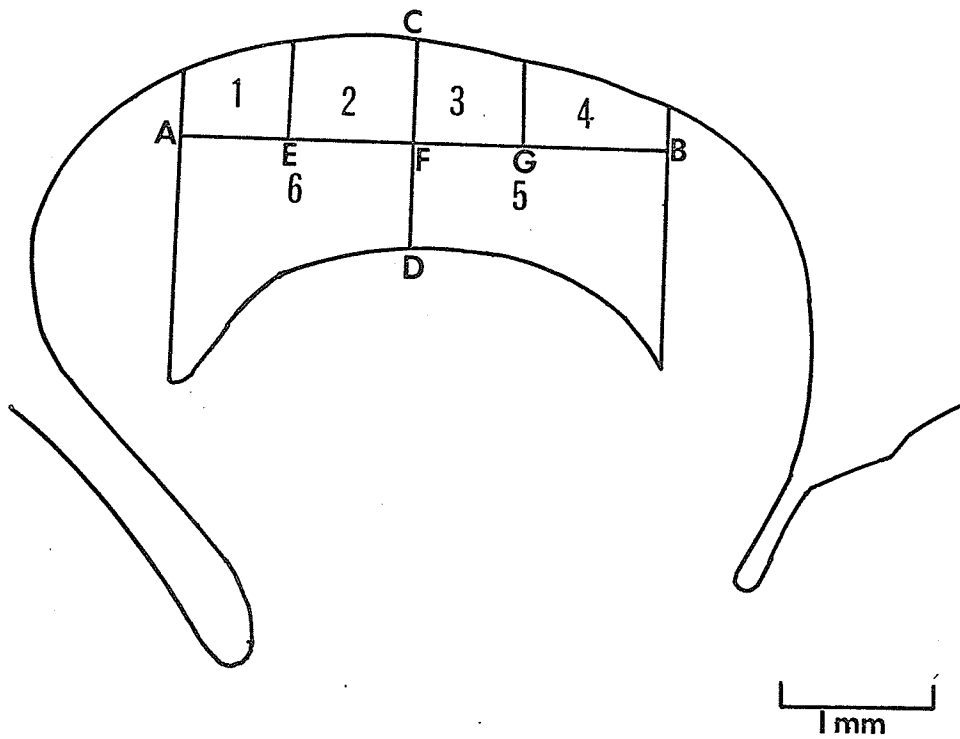


Figure 2. Diagram to Show Sub-Divisions of Non-Operated Supra-Sylvian Gyrus into 6 Segments.

Segments 1, 2, 3 and 4 are of Equal Width.

Segments 5 and 6 are of Equal Width.

A-B Divides Cortical depth into Two Halves.

C-D Divides Cortical width into Two Halves.

A-E is equal in width to, E-F, F-G, G-B.

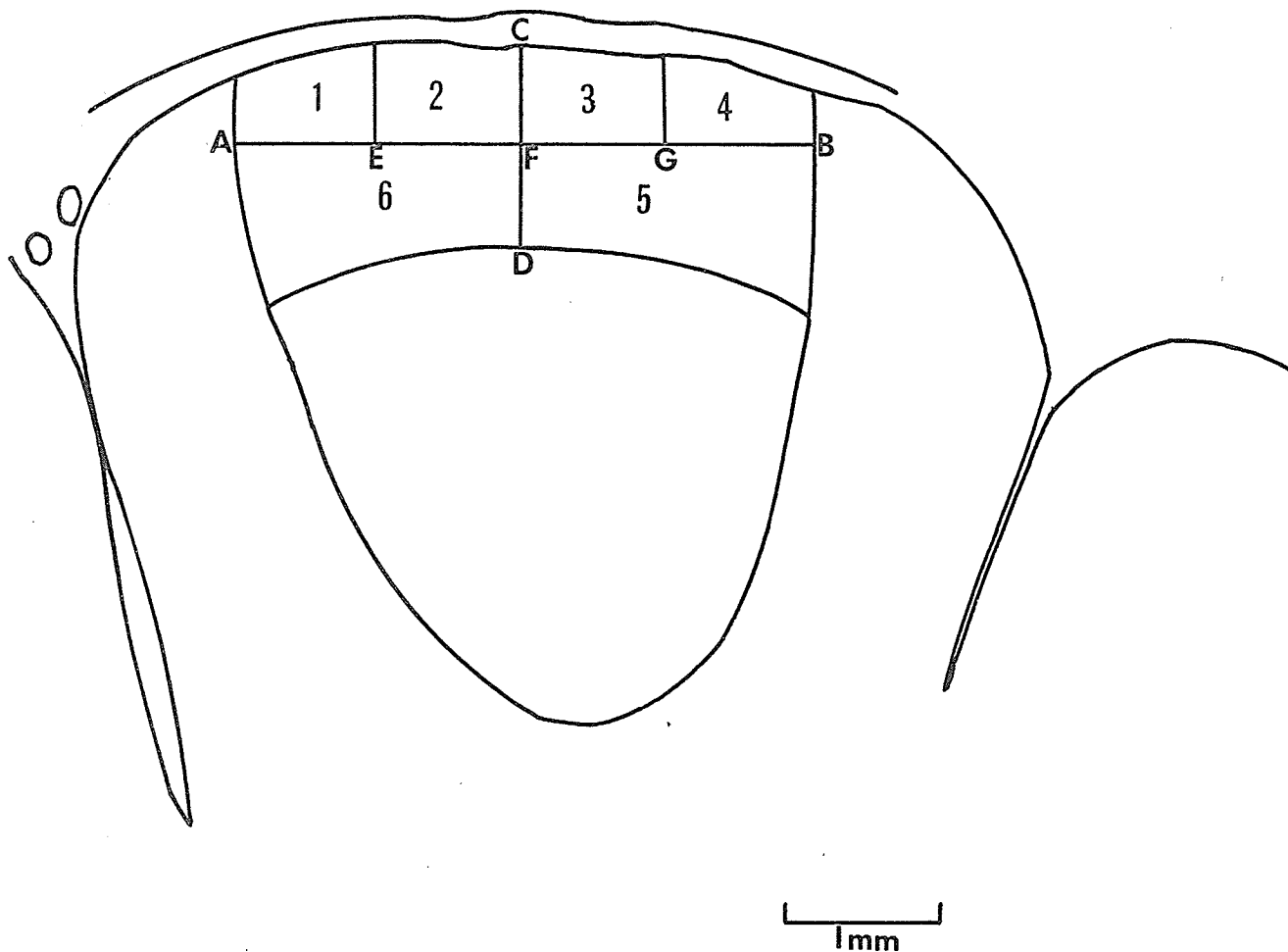


Figure 3. Diagram to Show Sub-Divisions of Acutely Isolated Supra-Sylvian Gyrus into 6 Cortical Segments.

Segments 1, 2, 3 and 4 are of Equal Width

Segments 5 and 6 are of Equal Width.

A-B Divides Cortical depth into Two Halves.

C-D Divides Cortical width into Two Halves.

A-E is equal in width to, E-F, F-G, G-B.

Chronically Isolated Cortex. Coronal sections of chronically isolated cortex were sub-divided into six cortical segments by methods similarly described for acutely isolated cortex. All segments were measured within the chronic slab as shown in (Figure 4).

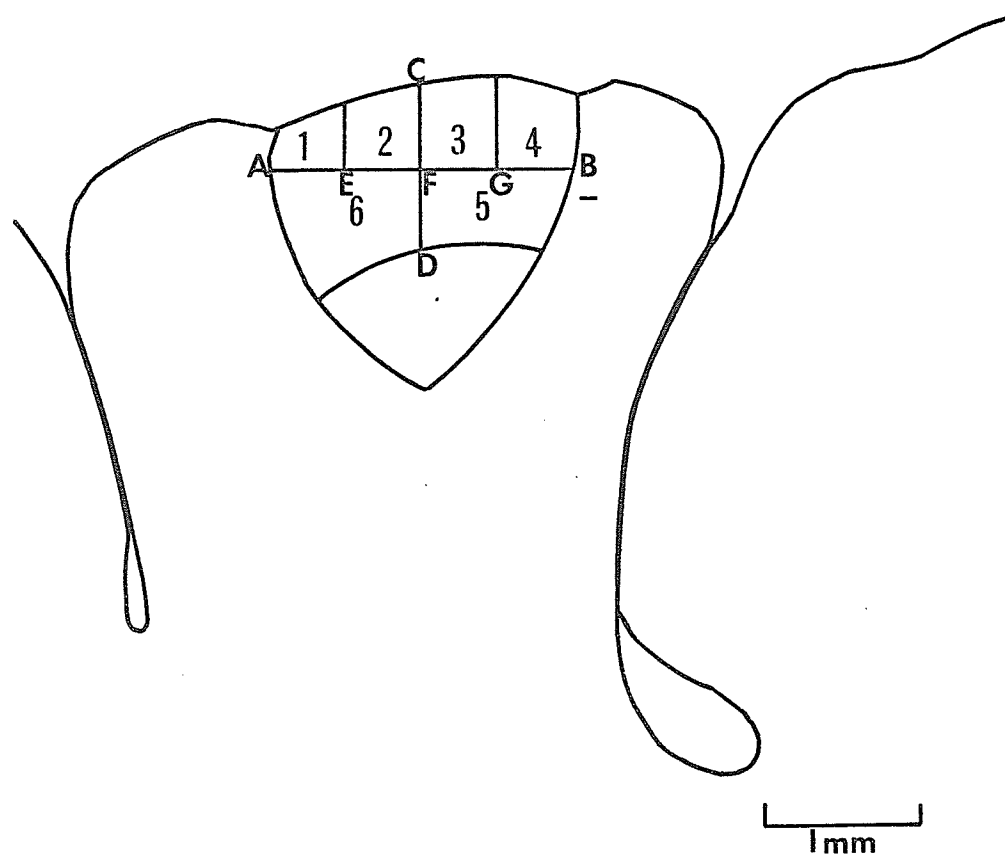


Figure 4. Diagram to Show Sub-Divisions of Chronically Isolated Cortex into 6 Segments.

Segments 1, 2, 3 and 4 are of Equal Width.

Segments 5 and 6 are of Equal Width.

A-B Divides Cortical depth into Two Halves.

C-D Divides Cortical width into Two Halves.

A-E is equal in width to, E-F, F-G, G-B.

Astrocytic Population Density. Astrocytic population densities were ^{calculated} at two millimeter intervals and at the mid-point along the length of intact and isolated cortex.

The calculations required for this study were as follows:

(a) volume of each cortical segment and (b) the number of observable protoplasmic astrocytes lying within each cortical segment. The volume of cortical tissue in each segment was calculated from the number of micrometer squares in each cortical segment multiplied by the thickness of the section.

Sample Calculation. At X50 magnification each square on the ocular insert measured 300 x 300 microns. The thickness of the section was 40 microns. Therefore the volume of tissue in each square was:

$$(300 \times 300 \times 40) \text{ } \mu^3$$

For "n" number of squares the volume (V) of tissue

$$= (n \times 300 \times 300 \times 40) \text{ } \mu^3$$

$$= \frac{(n \times 300 \times 300 \times 40)}{10^9} \text{ mm}^3$$

Calculated Astrocytic Density (D) for each segment:

$$D = \left(\frac{\text{Number of Observable Cells}}{\text{Volume of Tissue}} \right) \text{ mm}^3$$

Statistical Analysis. Determination of statistical significance was carried out by paired comparison "t" test and student's "t" test. Paired comparison "t" test was used to determine statistical significance between segments of the gyri. In comparisons between different gyri of the same and different cats student's "t" test was used. All means with their standard errors are given for individual segments in a representative coronal section for each type of examined. P values were obtained from a two tailed "t" table (Fisher and Yates 1953).

RESULTS

1. ANATOMICAL FEATURES

Gross Observations.

Intact Cortex. Gross examinations of intact supra-sylvian gyri showed that they were similar in colour to the adjacent marginal and ectosylvian gyri. Small blood vessels beneath the pia-mater did not appear swollen nor collapsed following craniectomy. None of the intact gyri examined in this study was found to have any kind of inherent malformation or gross pathology. Visual comparisons between supra-sylvian gyri in the same cat and between different cats showed slight variations in shape and size. In most cases the anterior and posterior terminations of the gyrus were not clearly defined. The ansatus sulcus which is situated at the anterior region of the gyrus showed several variations in angular pattern. The posterior termination of the supra-sylvian gyrus was taken as that point where the lateral and posterior sulci appeared to meet.

Acutely Isolated Cortex. The supra-sylvian gyrus in which an acutely isolated slab had been prepared showed indications of slight swelling. The edematous swelling was probably the result of trauma following surgical procedures during the preparation of the cortical slab. No attempt was made to calculate the increase in volume of

cortical tissue following brain edema.. Extensive studies dealing with the increase of cortical depth and width in acutely isolated cortices have been previously reported by Reiffenstein (1964), Weisman (1969), and Krnjevic et al (1970). Morphogenic variations, similar to those described for intact supra-sylvian gyri, were found also in acutely isolated gyri. Pial blood vessels did not show any signs of luminal collapse. Cortical side cuts were observed as superficial dark lines on the pia-mater in the form of a rectangle. These lines were used to locate accurately the position of the acutely isolated slab prior to excision of the gyrus.

Chronically Isolated Cortex. During the period of cortical chronicity in the left supra-sylvian gyrus, newly formed bony tissues were found to develop over the surgically craniectomized area of the parietal bone. The newly regenerated bone showed a mottled surface and was considerably thinner than the surrounding intact bony tissue. Connective tissue had formed fibrous adhesions between the bone and dura-mater as well as between dura and pia-mater. During the operation it was possible to separate the fibrous adhesions without damaging the underlying cortex of the supra-sylvian gyrus. Visual observations of the gyrus in which a chronic slab had been prepared showed evidences of cortical reduction in volume.

Blood vessels observed in the pia-mater of all

non-operated chronic homotopes were regionally comparable to those found on left supra-sylvian gyri. Gross morphogenic variations found on chronic homotopes were similar to those found on left supra-sylvian gyri. Gross morphogenic variations found on chronic homotopes were similar to those previously described for intact gyri.

2. MICROSCOPIC OBSERVATIONS OF HISTOLOGICAL PREPARATIONS

Intact Gyri. Frozen coronal sections of intact supra-sylvian gyri, when viewed under light microscopy, appeared yellow in colour. The pial-surface of intact gyri was convex in shape and lightly impregnated with silver. Plate I

The ability to impregnate astrocytic cells was found to be most reliable using techniques described by Ramon-Moliner(1957). Other methods, such as Cajal's Gold Sublimate and Penfield's neuroglial technique, were observed to randomly impregnate astrocytic cells in a non-uniform pattern. The percentage of astrocytic cells impregnated by Ramon-Moliner's (1957) technique was calculated for this study to be approximately 1.5%. This rate of astrocytic cell impregnation compared favourably with calculations obtained using a standard histological technique of formalin fixed intact cerebral cortex which was infiltrated with paraffin and stained by cresyl violet. Protoplasmic astrocytes observed in the cerebral cortex of the supra-sylvian gyri have a mossy appearance and give rise to short darkly stained

protoplasmic processes(Plate II). These processes were observed to emerge from the perikaryon. The terminal end feet of protoplasmic astrocytic processes were observed to be intimately associated with blood-capillaries and neurons. (Plate II andIII).

In the white matter fibrous astrocytes appeared in large numbers. They were noted to have long fibrous processes which extended from the perikaryon for distances up to 500 microns. Fibrous astrocytes were also observed to make intimate contacts with blood vessels by means of their terminal processes.

Only a few oligodendrocytes and microcytes were observed to be impregnated and were randomly distributed throughout the cerebral cortex. No effort was made to estimate the population of these oligodendrocytes and microcytes. A few neurons were found to be impregnated throughout the cortical tissues. Those neurons which were impregnated in the lower cortical layers showed a darkly stained perikaryon on which an apical dendritic shaft and a few basilar dendrites. It was not possible by means of this stain technique to make a detailed study of the neurons.

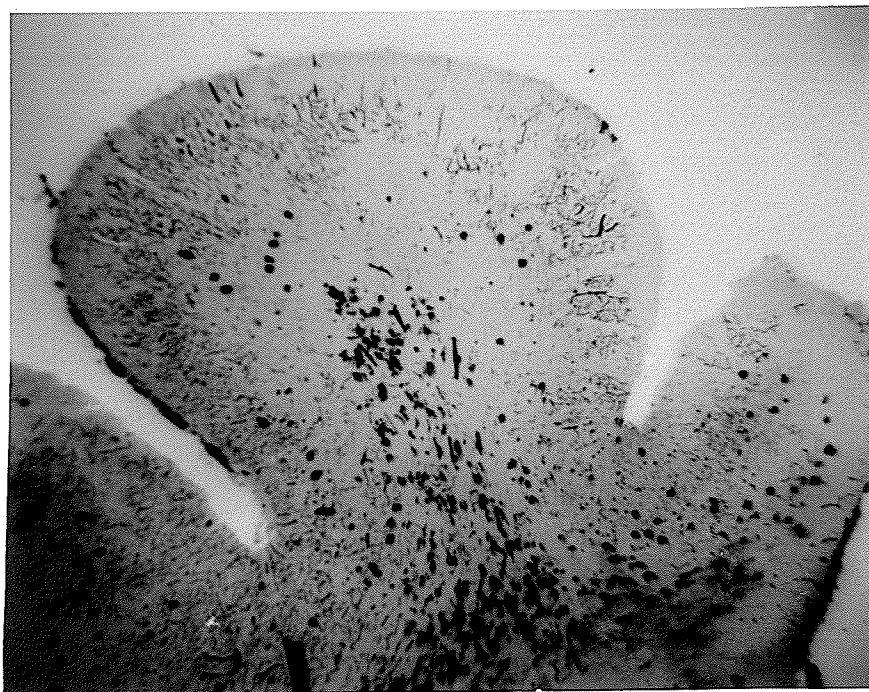


Plate 1. Photo-micrograph to show coronal section of non-operated supra-sylvian gyrus stained according to Ramon-Moliner (1957) technique. X6.

Dendrite

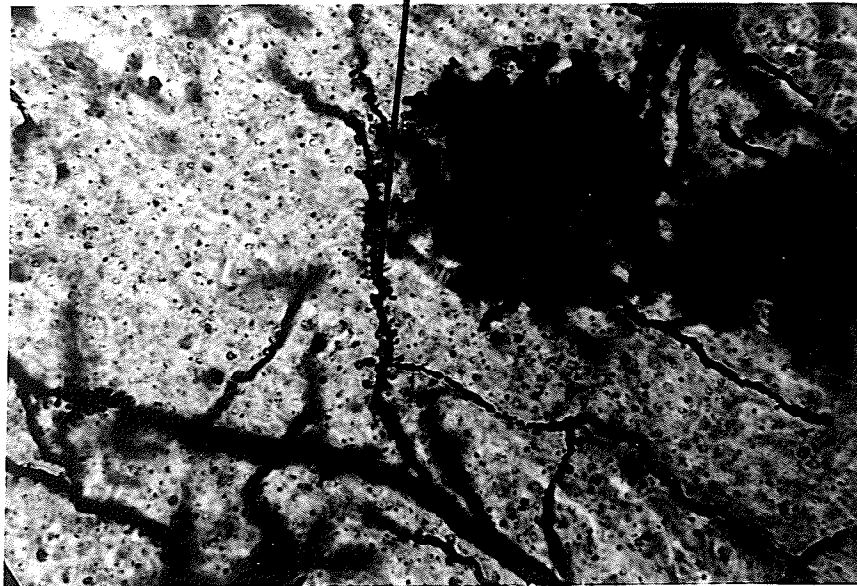


Plate II. Photo-micrograph to show relationship between protoplasmic astrocyte and dendrite. X1000.

Blood Capillary

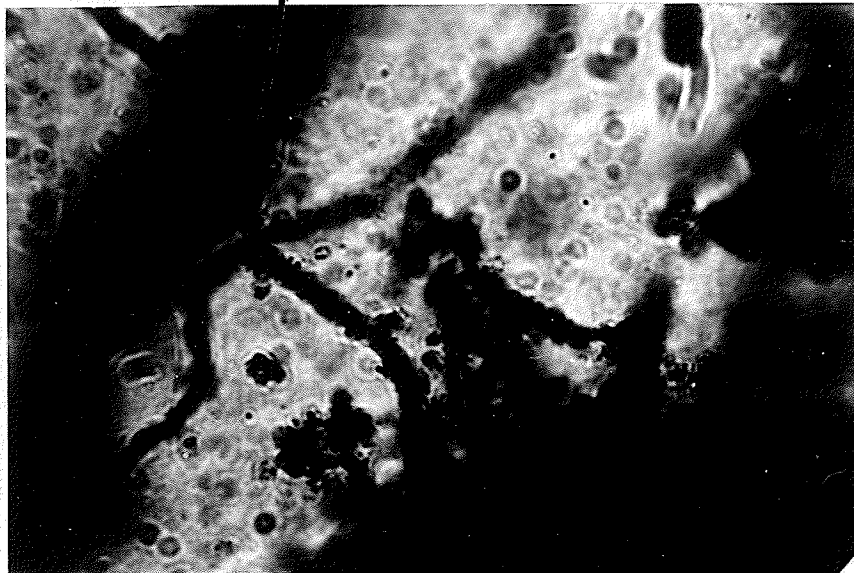


Plate III. Photo-micrograph to show relationship between
protoplasmic astrocyte process and blood vessel.
X1000.

Acutely Isolated Cortex. Coronal sections of acutely isolated cortex showed clearly the two side cuts made for neuronal isolation. The scar was moderately impregnated with silver (Plate IV). An exudate consisting of blood-cells and clear fluid was observed on both sides of the side cuts as well as the under-cut. Rate of impregnation of astrocytic cells was similar to that described for intact gyri.

The effects of short term isolation on the histological rearrangement of the supra-sylvian gyrus of the cat have been previously reported by Weisman (1967). In this study coronal sections showed that the acutely isolated cortex had swollen slightly when compared to intact gyri. Bi-lateral structural deformation of acutely isolated slabs was not observed to be adjacent to the undercut of the white matter and these spaces were filled with blood-cells and a clear fluid. These observations confirmed the findings reported earlier by Weisman et al (1967).

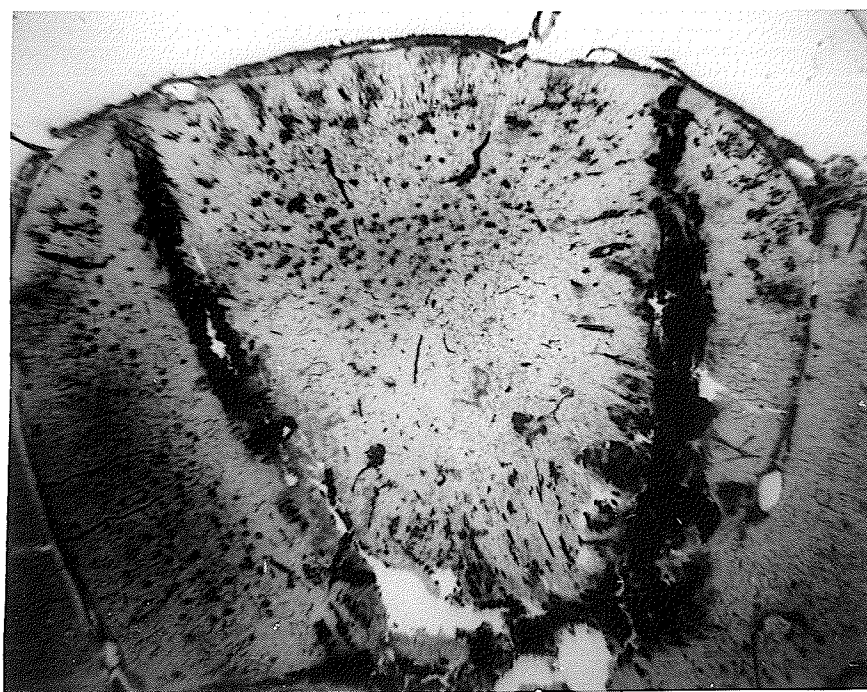


Plate IV. Photo-micrograph to show coronal section of acutely isolated cortex stained according to Ramon-Moliner (1957) technique. Picture shows the isolated part of the supra-sylvian gyrus. X2.

Chronically Isolated Cortex. Protoplasmic astrocytes were clearly impregnated in chronically isolated Cortex. The astrocytes appeared somewhat small in diameter than their counterparts in intact cortex. No attempt was made to measure the sizes of the cells. The isolated slab was triangular in shape and reduced considerably in volume. (Plate V). The histological reorganization of chronically isolated cortex as reported by others was found to be in agreement with the histo-anatomical findings observed in this study.



Plate V . Photo-micrograph to show coronal section of chronically isolated cortex stained according to Ramon-Moliner (1957) technique. Picture shows the isolated part of the supra-sylvian gyrus. X6.

3. ASTROCYTIC CELL DENSITY

Intact Gyrus. The astrocytic population densities were calculated at 2 mm intervals in 8 intact gyri comprising 4 intact left and their 4 intact homotopic gyri. The results of these calculations are shown in Table 1 and in the form of three dimensional histograms in figs. 5 and 6. Paired comparison tests were carried out in the intact gyri to test for significant differences between the following cortical segments:

(a) Cortical segment 1 vs 4; (b) 2 vs 3; (c) 5 vs 6.

The tests were repeated in the intact homotopic gyri. The results showed that there were no significant differences in populations densities at each "2" mm intervals, along the length of both intact left and homotopic gyri for each of the above tests performed.

Paired comparison tests were made also for significant differences in population densities between the upper and lower cortical halves. A highly significant difference ($P \leq 0.01$) was found to occur between the upper and lower cortical segments at each "2" mm intervals along the entire length of both left and right gyri. However, there was no significant difference between the upper and lower half at "0" mm point in the left gyri. These differences are graphically shown in figs. 5 and 6. No significant differences in densities were found to occur between similar

TABLE 1

Mean astrocytic population densities in 4 intact left gyri.

Distance from Ant. End in mm	Observed Astrocytes					
	Cortical Segments					
	1	2	3	4	5	6
0	208	59.5	159	54	189	160
2	138	111.1	163	63.5	337	446
4	0	61.5	0	0	268	391
5	17	49.0	42	102	263	331
6	0	119	169	25	408	300
8	17	77	77	17	249	364
10	121	103	110	41	391	369

Mean astrocytic population densities in 4 intact homotopic gyri.

0	64	143	124	166	358	319
2	345	277	297	269	319	504
4	159	168	168	104	351	365
5	34	29	99	100	356	388
6	170	89	49	192	369	411
8	152	79	119	107	522	444
10	97	119	126	17	402	384

LEFT INTACT

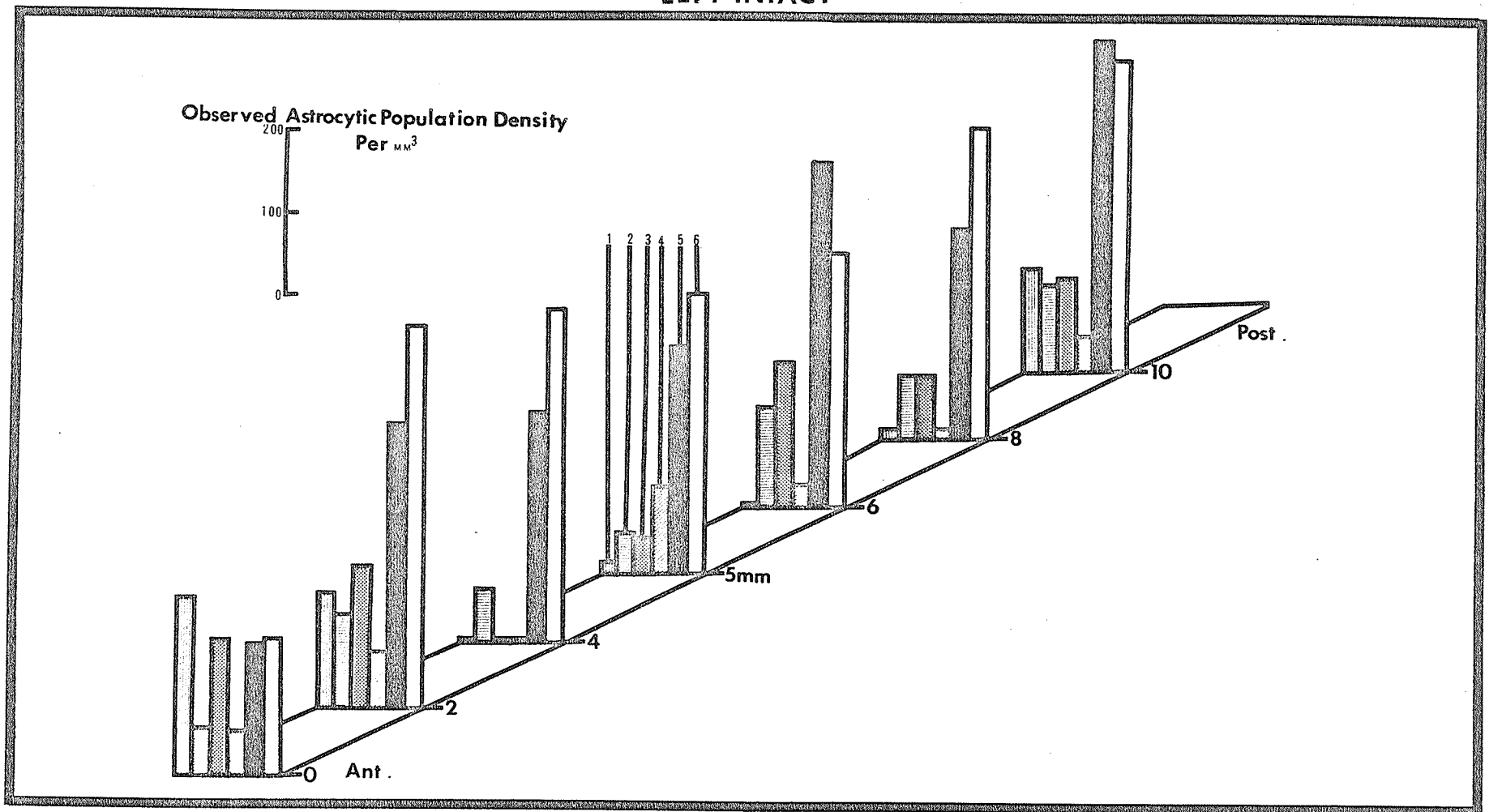


Figure 5. Astrocytic population densities calculated at "2" mm intervals in 4 intact supra-sylvian gyri. The anterior and posterior regions of the gyrus are indicated at "0" mm and "10" mm.

INTACT HOMOTOPE

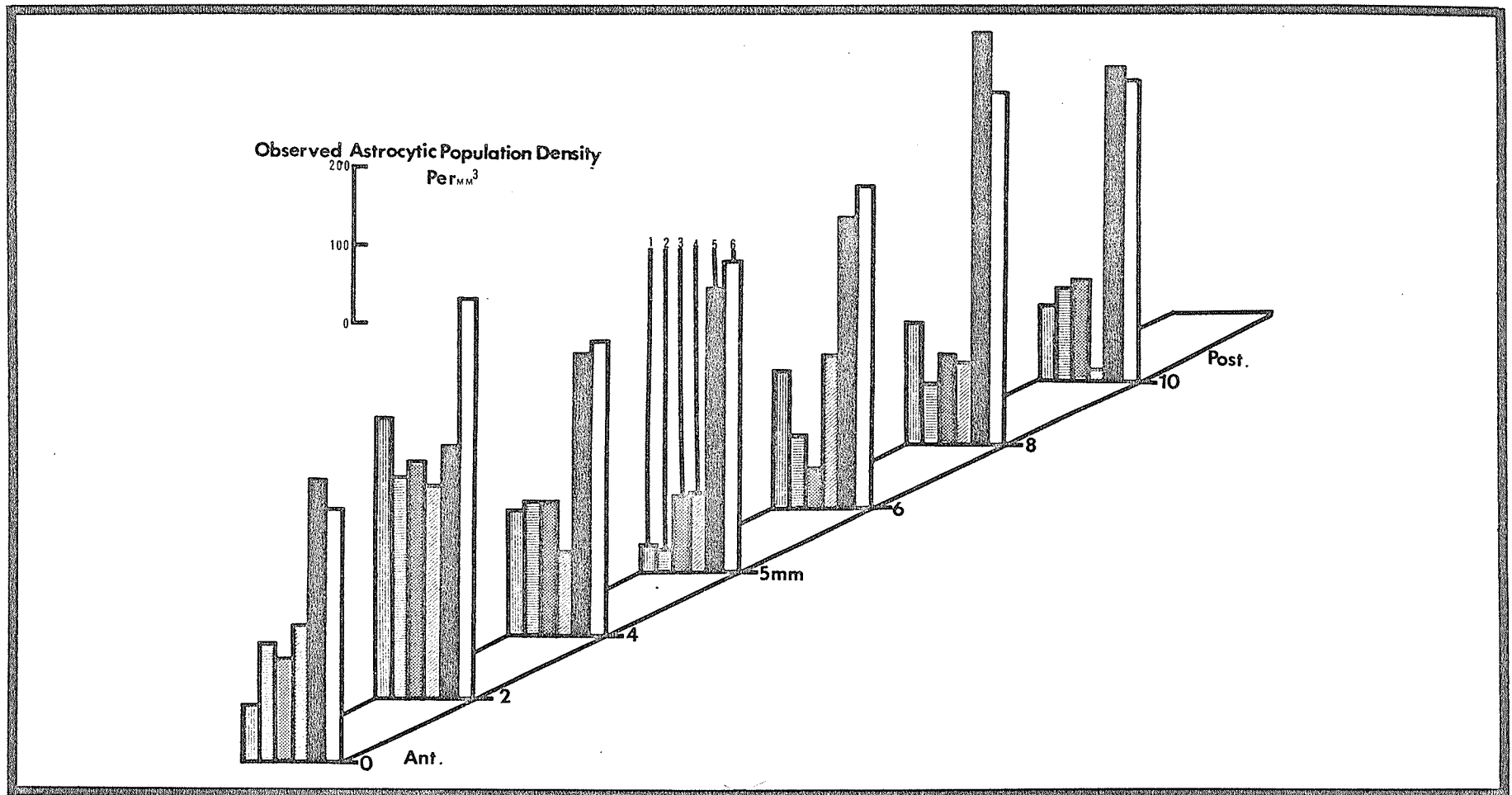


Figure 6. Astrocytic population densities calculated at "2" mm intervals in 4 intact supra-sylvian gyri. The anterior and posterior regions of the gyrus are indicated at "0" mm and "10" mm.

segments at "2" mm intervals in both left and right gyri. However, slight differences in densities do exist between similar segments which were found to be non-significant.

The pooled densities from segments 5 and 6 at each 2 mm interval in the left intact gyri were compared to similar cortical segments in the intact homotopic gyri. No significant differences were found to exist between the compared pooled segments along the entire lengths of the gyri. Although no significant differences were found to exist between cortical segments 1 and 4, 2 and 3 in the upper half and between 5 and 6 in the lower cortical halves in both left and right intact gyri, nevertheless, the individual differences found at each 2 mm intervals are real.

The data from all similar cortical segments in all the intact left gyri were pooled and the mean astrocytic population density was calculated and is shown in the form of a schematic coronal section in Fig. 7, and as a histogram in Fig. 8. Population densities and standard errors for each cortical segment are shown. No significant differences in population densities were found to occur between segments 1 and 4, 2 and 3, 5 and 6 in the pooled intact left gyri.

1	2	3	4
$N = 28$ $\bar{X} = 75.28$ $S = 119.90$ $SE = 22.60$	$N = 28$ $\bar{X} = 78.28$ $S = 75.77$ $SE = 14.32$	$N = 28$ $\bar{X} = 107.78$ $S = 90.98$ $SE = 17.18$	$N = 28$ $\bar{X} = 43.78$ $S = 67.70$ $SE = 12.79$
6		5	
$N = 28$ $\bar{X} = 344.89$ $S = 198.32$ $SE = 37.48$		$N = 28$ $\bar{X} = 333.25$ $S = 172.26$ $SE = 32.56$	

Figure 7. Mean astrocytic population densities in
4 intact left gyri.

N = Number of Histological Sections.

$\bar{X}$ = Mean Astrocytic Density.

S = Standard Deviation.

SE = Standard Error.

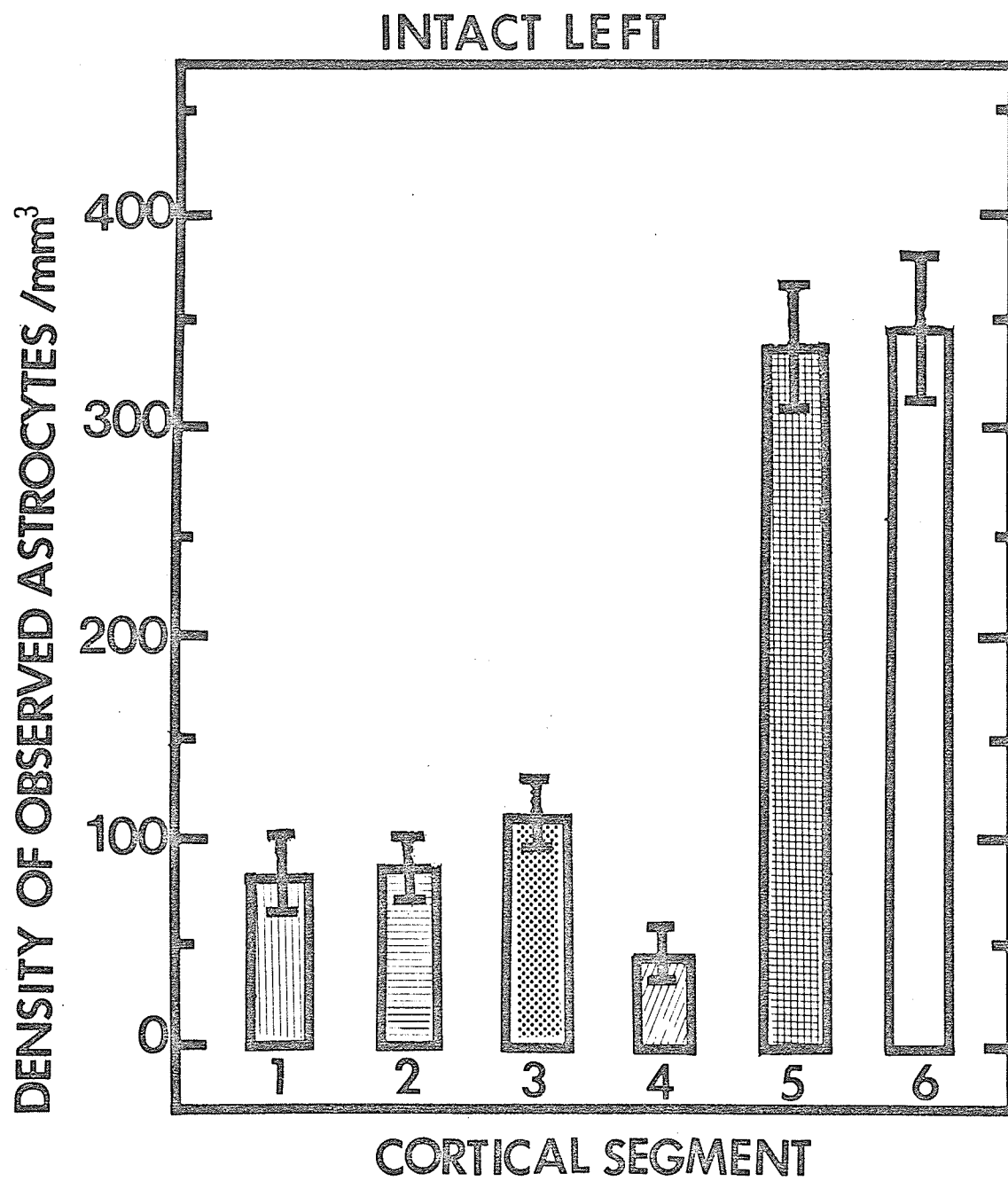


Figure 8. Mean astrocytic population densities in 4 intact left gyri.

The data from all the intact homotopic gyri were pooled and calculated for population densities as previously described for all the pooled intact left gyri. The results of these calculations are shown in fig. 9 and 10. No significant differences in population densities were found to occur between segments 1 and 4, 2 and 3, 5 and 6 in the pooled intact homotopic gyri.

The pooled cortical segments from the intact left gyri were compared by student "t" tests to similarly pooled cortical segments of intact homotopic gyri in the following manner:

left (1 plus 4) vs right (1 plus 4)

left (2 plus 3) vs right (2 plus 3)

left (5 plus 6) vs right (5 plus 6)

No significant differences were found to occur in any of the above tests. The mean astrocytic population density was significantly greater ($P \leq 0.001$) in the lower cortical half when compared to the upper cortical half in each of the pooled data from intact left and right gyri. No significant differences were found to exist between pooled data (1+2+3+4+5+6) of intact left gyri and pooled data (1+2+3+4+5+6) of intact homotopic gyri.

1	2	3	4
$N = 28$	$N = 28$	$N = 28$	$N = 28$
$\bar{X} = 139.14$	$\bar{X} = 129.53$	$\bar{X} = 145.28$	$\bar{X} = 129.60$
$S = 177.71$	$S = 146.43$	$S = 161.44$	$S = 173.68$
$SE = 33.59$	$SE = 27.68$	$SE = 30.57$	$SE = 32.83$

6	5
$N = 28$	$N = 28$
$\bar{X} = 403.10$	$\bar{X} = 383.75$
$S = 256.85$	$S = 256.68$
$SE = 48.55$	$SE = 48.52$

Figure 9. Mean astrocytic population densities in 4 intact homotopic gyri.

N = Number of Histological Sections.

$\bar{X}$ = Mean Astrocytic Density.

S = Standard Deviation.

SE = Standard Error.

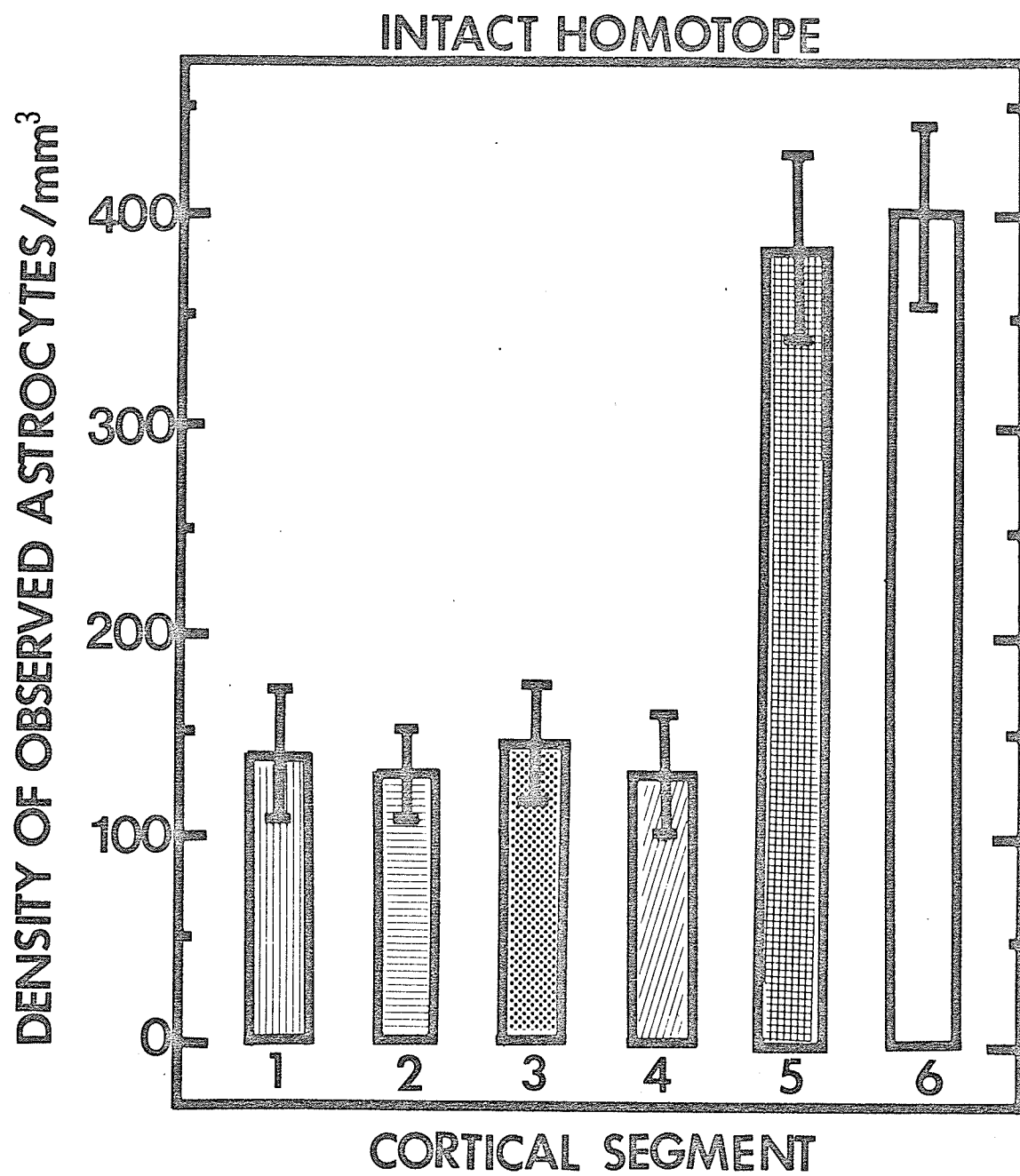


Figure 10. Mean astrocytic population densities in 4 intact homotopic gyri.

Acutely Isolated Cortex. Astrocytic population density studies were made at 2 mm intervals from 3 acutely isolated left cortical slabs and their 3 acutely isolated homotopic cortical slabs. All acute slabs were electrically stimulated by surface stimulating electrodes as outlined in the methods section of this thesis.

The population densities for each of the cortical segments are shown in Table 2 and in the form of three dimensional histograms in Figs. 11 and 12. Statistical analyses for significance were carried out between cortical segments in acute slabs by methods described previously for non-operated intact cortex.

Paired comparison tests were carried out to determine whether any significant changes occurred in the astrocytic population density as a result of short-term isolation accompanied by electrical stimulation.

No significant differences were found to exist between cortical segments: (a) 1 and 4; (b) 2 and 3; (c) 5 and 6 at "2" mm intervals for the entire length in each of acutely isolated left and their acutely isolated homotopic gyri.

The data of all cortical segments in the upper cortical half of each "2" mm interval were pooled, i.e. 1+2+3+4, and the population density compared to other similarly pooled cortical segment within the same slab. No significant

TABLE 11

Mean astrocytic population densities in 3 acutely isolated left gyri.

Distance from Ant. End in mm	Observed Astrocytes					
	Cortical Segments					
	1	2	3	4	5	6
0	0	83.3	111	69.3	257	391
2	37	63.3	0	37.0	351	172.3
4	55.3	79.3	52.6	0	260.6	167.6
5	37	33	26.3	37	184.6	208.3
6	37	263	92.3	37	166.3	222
8	248.3	137	142	89	363.6	391.6
10	44.8	39.6	165.6	60	2033	227.6

Mean astrocytic population densities in 3 acutely isolated homotopic gyri.

0	74	105	26.3	0	141.6	135.3
2	37	92	26.3	37	197	185
4	190	132	198	96	141.3	240
5	33.3	178.3	116	62	197	178
6	106.3	118.6	118.6	95.6	221.6	223.6
8	220	65.6	66	64.3	129	129.6
10	97	171.6	211.3	112	376	361.6

LEFT ACUTE

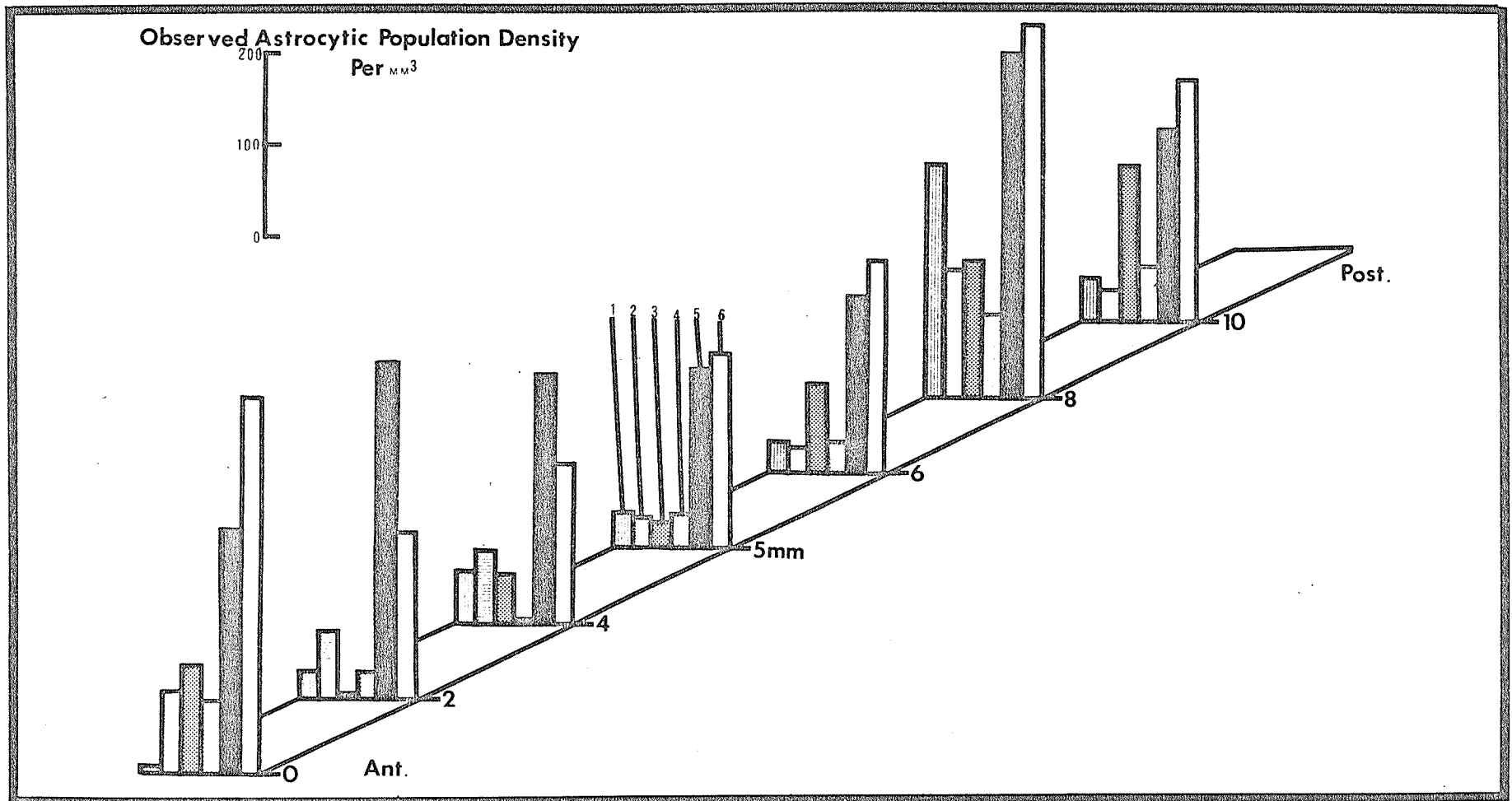


Figure 11. Astrocytic population densities calculated at "2" mm intervals in 3 acutely isolated homotopic slabs. The anterior and posterior regions of the gyrus are indicated at "0" mm and "10" mm.

ACUTE HOMOTOPE

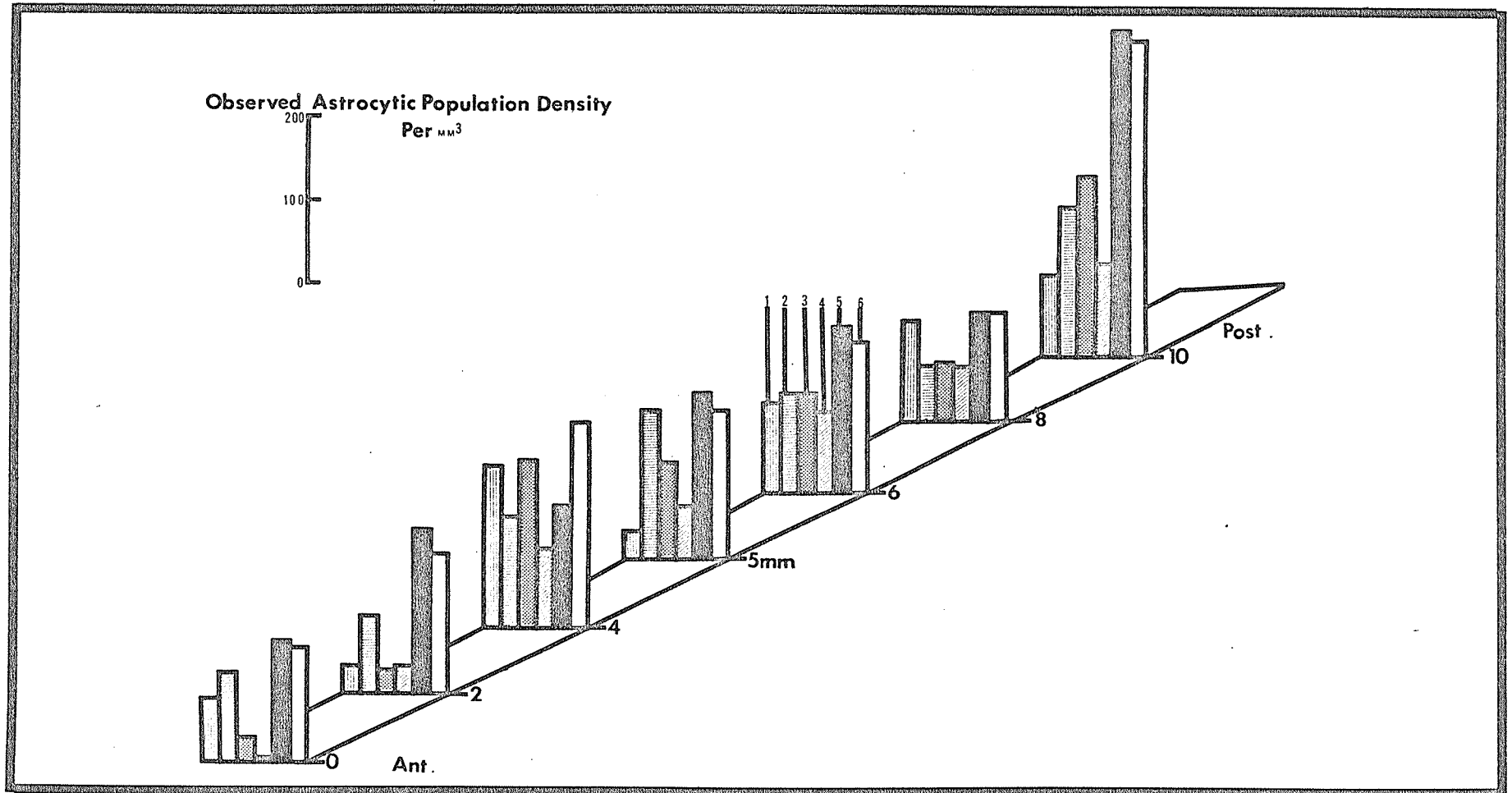


Figure 12. Astrocytic population densities calculated at "2" mm intervals in 3 acutely isolated slabs. The anterior and posterior regions of the gyrus are indicated at "0" mm and "10" mm.

differences in population density was found to occur between the compared areas. In the lower cortical half of the left acute slab only the "0" and "8" mm intervals were significantly greater than other similarly adjacent areas in the same slab. In the acute homotope slab the density at the "10" mm interval in the lower cortical half was significantly greater than other similar areas.

The astrocytic population density from the pooled data from all upper cortical segments (1+2+3+4) in 3 acutely isolated slabs was significantly less when compared to the population density from pooled data (5+6) in the lower cortical half ($P \leq 0.01$). Similar tests for significance were carried out for the homotopic gyri in which the density in the upper cortical half was found to be significantly less than the density in the lower cortical half ($P \leq 0.05$).

Additional tests for significance showed that there were no significant differences in density between pooled data in the following: (a) all left acutely isolated upper cortical segments and all acutely isolated homotopic upper cortical segments; (b) all left acutely isolated lower cortical segments and all acutely isolated homotopic lower cortical segments; (c) non-protected left acutely isolated cortical segments (1+4) and non-protected acutely isolated homotopic (1+4) segments; (d) surgically protected left acutely isolated cortical segments (2+3) and surgically protected acutely isolated homotopic (2+3) segments; (e) lower left acutely isolated cortical segments (5+6) and acutely isolated homotopic

(5+6) segments.

The pooled astrocytic population density was found to be slightly less in the lower cortical segments of acutely isolated slabs when compared by significance tests to the pooled lower cortical segments in each of intact left, intact homotope and non-operated chronic homotope. However, no significant differences in density were found between pooled data in the upper cortical halves of acutely isolated and non-operated cortices.

The data from all segments in all left acute slabs were pooled and are shown as a representative coronal section in schematic form in Fig. 13 and in histogram form in Fig. 14. Mean astrocytic population densities and standard errors are recorded for each of the six cortical segments. The data from the 3 homotopic acute slabs were similarly calculated and are shown in Figs. 15 and 16.

1	2	3	4
$\frac{N}{X} = 21$ $\bar{X} = 65.62$ $S = 124.49$ $SE = 27.17$	$\frac{N}{X} = 21$ $\bar{X} = 69.76$ $S = 80.12$ $SE = 17.48$	$\frac{N}{X} = 21$ $\bar{X} = 78.61$ $S = 101.64$ $SE = 22.18$	$\frac{N}{X} = 21$ $\bar{X} = 47.05$ $S = 68.85$ $SE = 15.62$

6	5
$\frac{N}{X} = 21$ $\bar{X} = 254.38$ $S = 182.88$ $SE = 39.91$	$\frac{N}{X} = 21$ $\bar{X} = 223.71$ $S = 106.23$ $SE = 23.18$

Figure 13. Mean astrocytic population densities in
3 left acutely isolated slabs.

N = Number of Histological Sections.

$\bar{X}$ = Mean Astrocytic Density.

S = Standard Deviation.

SE = Standard Error.

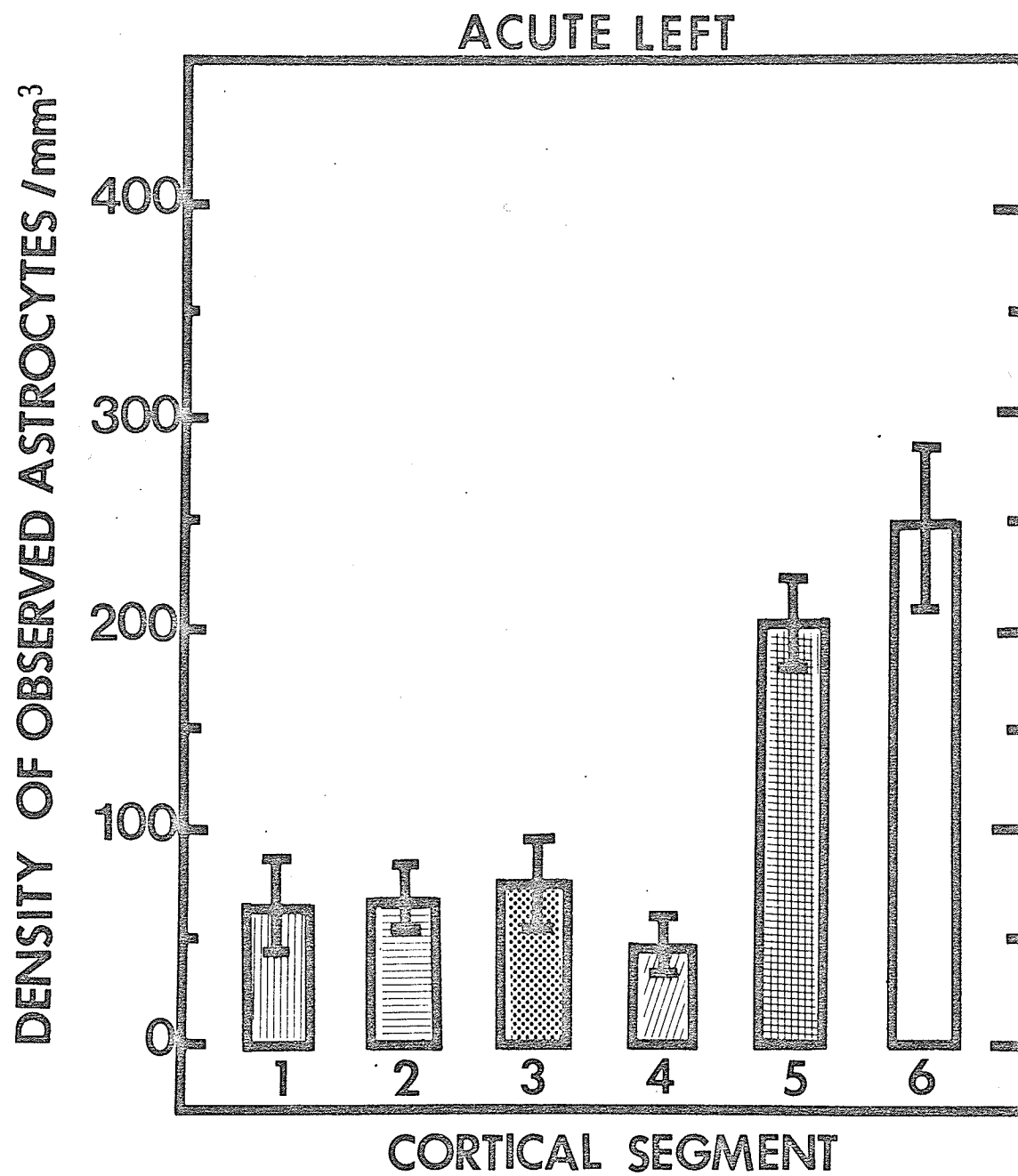


Figure 14. Mean astrocytic population densities in 3 left acutely isolated slabs.

1	2	3	4
N = 21	N = 21	N = 21	N = 21
$\bar{X}$ = 79.10	$\bar{X}$ = 118.66	$\bar{X}$ = 101.43	$\bar{X}$ = 66.71
S = 83.47	S = 122.81	S = 127.54	S = 70.54
SE = 18.21	SE = 26.80	SE = 27.83	SE = 15.39

6	5
N = 21	N = 21
$\bar{X}$ = 198.86	$\bar{X}$ = 200.52
S = 112.68	S = 117.24
SE = 24.59	SE = 25.58

Figure 15. Mean astrocytic population densities in
3 acutely isolated homotopic slabs.

N = Number of Histological Sections.

$\bar{X}$ = Mean Astrocytic Density.

S = Standard Deviation.

SE = Standard Error.

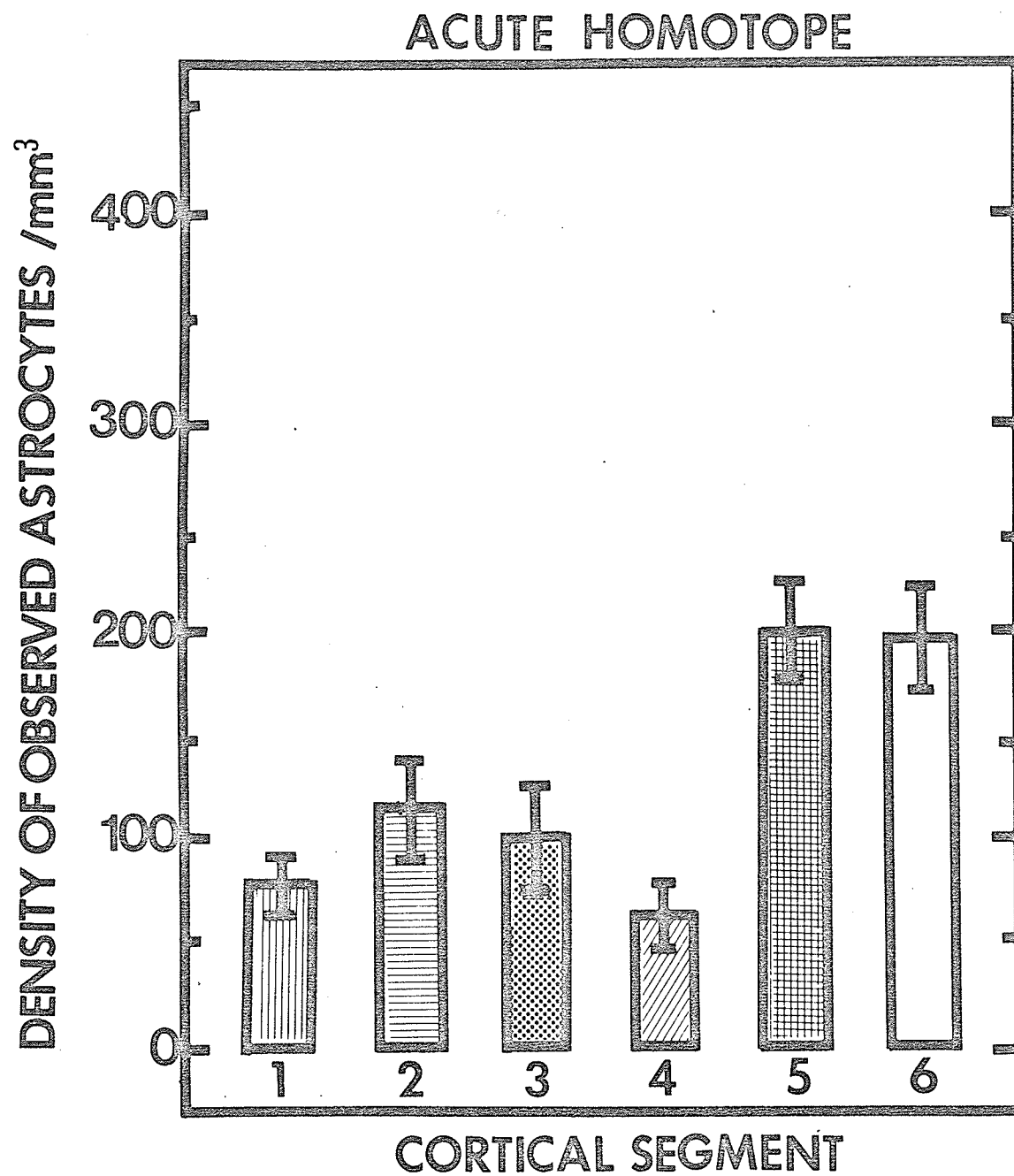


Figure 16. Mean astrocytic population densities in 3 homotopic acutely isolated slabs.

Chronically Isolated Cortex. The astrocytic population density was calculated at "2" mm intervals throughout the lengths of 3 chronically isolated slabs and their non-operated chronic homotopes. The results of these calculations are listed in Table 3. The density profiles are shown in 3 dimensional histograms in Figs. 17 and 18. Comparison tests for significance were carried out as previously described for intact and acutely isolated cortices

Paired comparison analyses throughout the length of chronic slabs showed that there were NO significant differences in density in the following: between each of the non-protected cortical segments 1 and 4 except at the 4 mm intervals; between each of the surgically protected cortical segments 2 and 3; between each of the lower cortical segments 5 and 6 except at the 10 mm intervals. In the non-operated chronic homotopes no significant differences in density were found to exist throughout the length of the gyri between the following cortical segments; 1 and 4; 2 and 3, 5 and 6.

TABLE 111

Mean astrocytic population densities in 3 chronically isolated left gyri.

Distance from Ant. End <i>mm</i>	Observed Astrocytes					
	Cortical Segments					
	1	2	3	4	5	6
0	259	182	268	319	413	320
2	217	180	193	163	394	308
4	378	110	39	23	264	312
5	110	88	177	96	251	482
6	148	78	57	125	222	315
8	179	144	259	166	303	338
10	333	244	187	144	621	394

Mean astrocytic population densities in 3 non-operated chronic homotopic gyri.

0	161	222	220	181	404	416
2	111	79	52	52	197	260
4	189	209	119	92	172	160
5	46	105	92	69	237	244
6	129	132	131	266	252	203
8	235	158	211	26	123	141
10	69	66	66	131	265	301

CHRONIC LEFT

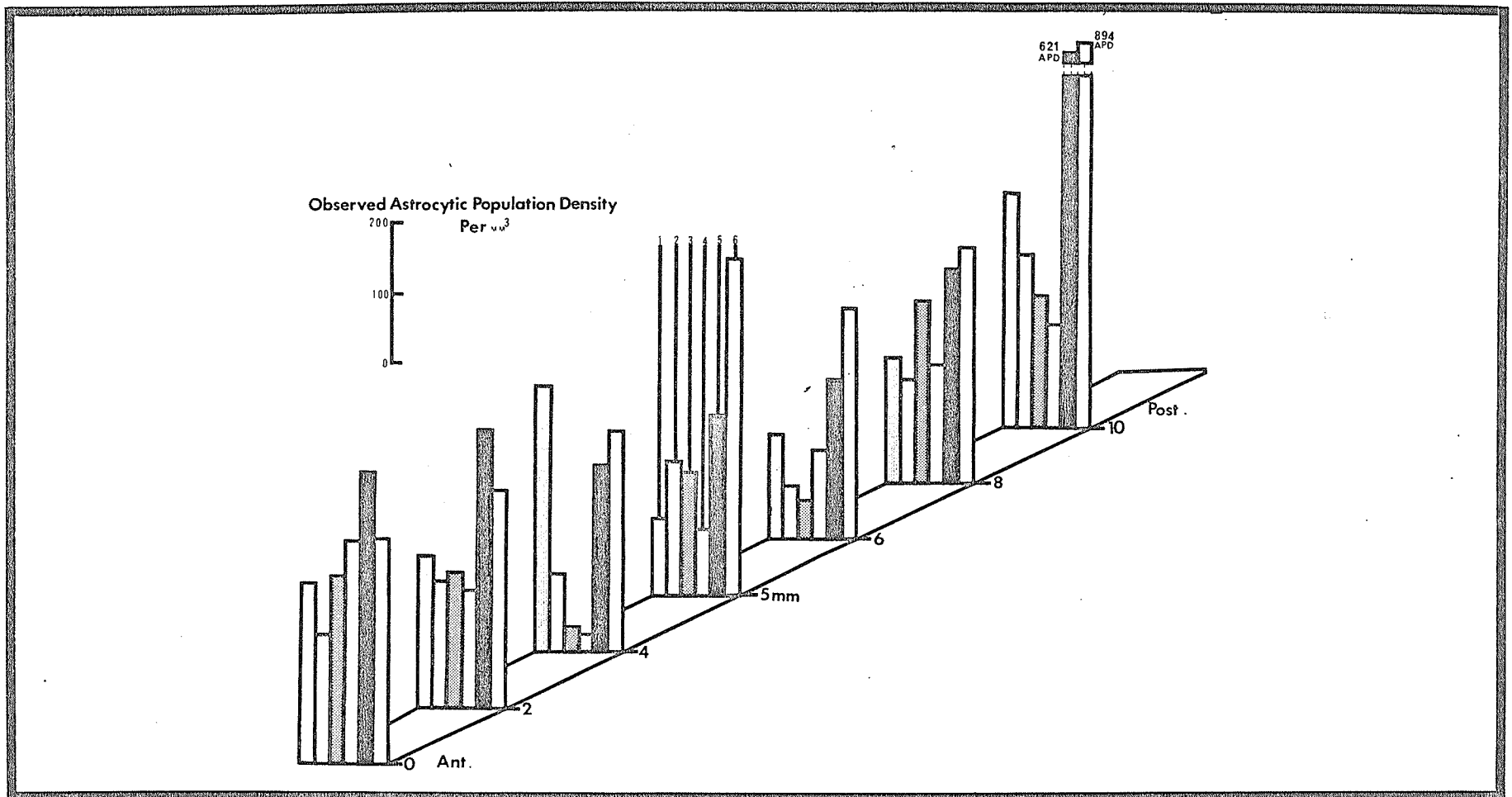


Figure 17. Astrocytic population densities calculated at "2" mm intervals in 3 chronically isolated left supra-sylvian gyri. The anterior and posterior regions of the gyrus are indicated at "0" mm and "10" mm.

CHRONIC HOMOTOPE

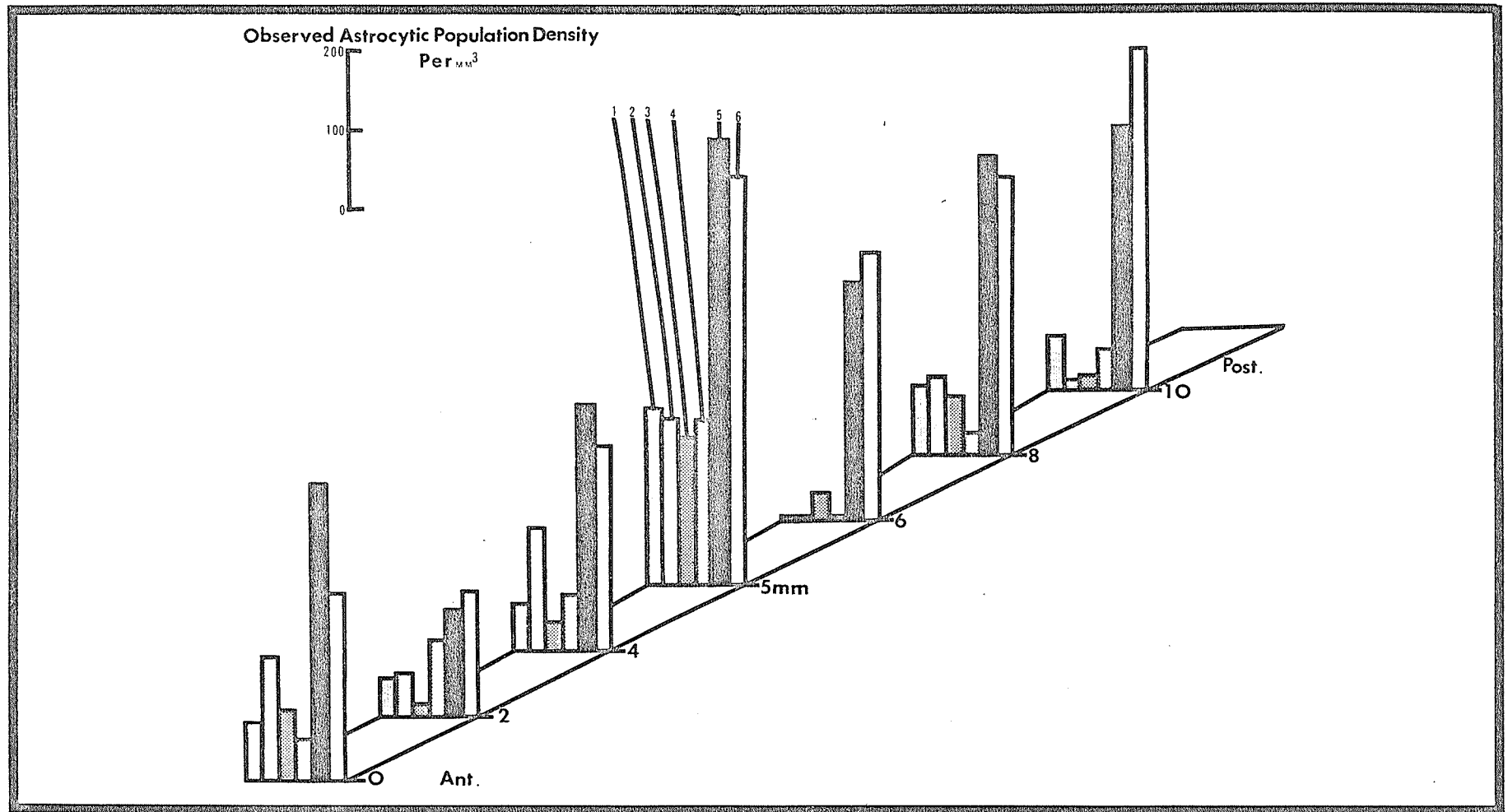


Figure 18. Astrocytic population densities calculated at "2" mm intervals in 3 chronic non-operated homotopic gyri. The anterior and posterior regions of the gyrus are indicated at "0" mm and "10" mm.

The astrocytic population density in chronically isolated slabs was compared to the density in non-operated chronic homotopes. The results showed that a highly significant increase in astrocytic population density had occurred in upper cortical segments of chronically isolated slabs ($P \leq 0.001$). The density in pooled cortical segments 1 plus 4 in chronic slabs was found to be significantly higher at each 2 "mm" interval when compared to similar segments in chronic homotopes ($P \leq 0.001$). Chronic segments 2 plus 3 were found also to be significantly greater when compared to similar cortical segments in chronic homotopes. The data from all upper cortical segments were pooled and the mean astrocytic population density was calculated for the 3 chronic slabs. The results were compared to similarly pooled data from chronic homotopes. The population density was significantly higher in chronic slabs than in chronic homotopes ($P \leq 0.001$) as shown in Figs. 19 and 20. Except at the 10 "mm" interval no significant differences in densities were found to occur between pooled segments (5+6) in chronic slabs and similarly pooled cortical segments in chronic homotopes. Direct microscopic observations, however, seemed to indicate that there was a tendency for the density in the lower cortical half of chronic slabs to be higher than corresponding areas of chronic homotopes.

The densities in chronically isolated slabs were compared with the densities in acutely isolated slabs. The

results showed that: (a) there was a significantly ($P \leq 0.001$) higher cell density in pooled chronic segments 1 plus 4 when compared to pooled acutely isolated segments 1 plus 4 in both left and right gyri; (b) there was a significantly ($P \leq 0.001$) higher cell density in pooled chronic segments 2 plus 3 when compared to pooled acutely isolated segments 2 plus 3 in both left and right gyri; (c) there was a significantly higher cell density in pooled chronic segments 5 plus 6 when compared to pooled acutely isolated segments 5 plus 6 in both left and right gyri ($P \leq 0.05$).

The densities of chronically isolated slabs were compared with the densities of non-operated cortices. The results showed that: (a) there was a significantly higher ($P \leq 0.05$) cell density in pooled chronic segments 1 plus 4, 2 plus 3, than in similar pooled segments in non-operated cortices; (b) there were NO significant differences between pooled chronic segments 5 plus 6 and pooled chronic homotopes 5 plus 6; (c) there were NO significant differences between pooled chronic segments 5 plus 6 and pooled segments 5 plus 6 of either intact left or intact homotope.

The mean astrocytic population density which would be found in a representative coronal section from pooled chronically isolated left gyri and pooled non-operated chronic homotopes are shown in Figs. 19 and 20 and in histogram form in Figs. 21 and 22.

1	2	3	4
N = 21	N = 21	N = 21	N = 21
$\bar{X}$ = 227.71	$\bar{X}$ = 146.95	$\bar{X}$ = 156.48	$\bar{X}$ = 148.19
S = 183.96	S = 83.36	S = 131.48	S = 155.19
SE = 40.14	SE = 18.19	SE = 28.69	SE = 33.87

6	5
N = 21	N = 21
$\bar{X}$ = 448.38	$\bar{X}$ = 353.05
S = 281.83	S = 248.58
SE = 61.50	SE = 54.25

Figure 19. Mean astrocytic population densities in 3 chronically isolated left supra-sylvian gyri.

N = Number of Histological Sections.

$\bar{X}$ = Mean Astrocytic Density.

S = Standard Deviation.

SE = Standard Error.

1	2	3	4
$N = 21$	$N = 21$	$N = 21$	$N = 21$
$\bar{X} = 80.19$	$\bar{X} = 76.81$	$\bar{X} = 60.62$	$\bar{X} = 60.62$
$S = 107.67$	$S = 91.35$	$S = 78.53$	$S = 101.70$
$SE = 23.50$	$SE = 19.93$	$SE = 17.14$	
6		5	
$N = 21$		$N = 21$	
$\bar{X} = 341.70$		$\bar{X} = 354.95$	
$S = 154.52$		$S = 147.80$	
$SE = 34.55$		$SE = 32.25$	

Figure 20. Mean astrocytic population densities in
3 chronic non-operated homotopic supra-
sylvian gyri.

N = Number of Histological Sections.

$\bar{X}$ = Mean Astrocytic Density.

S = Standard Deviation.

SE = Standard Error.

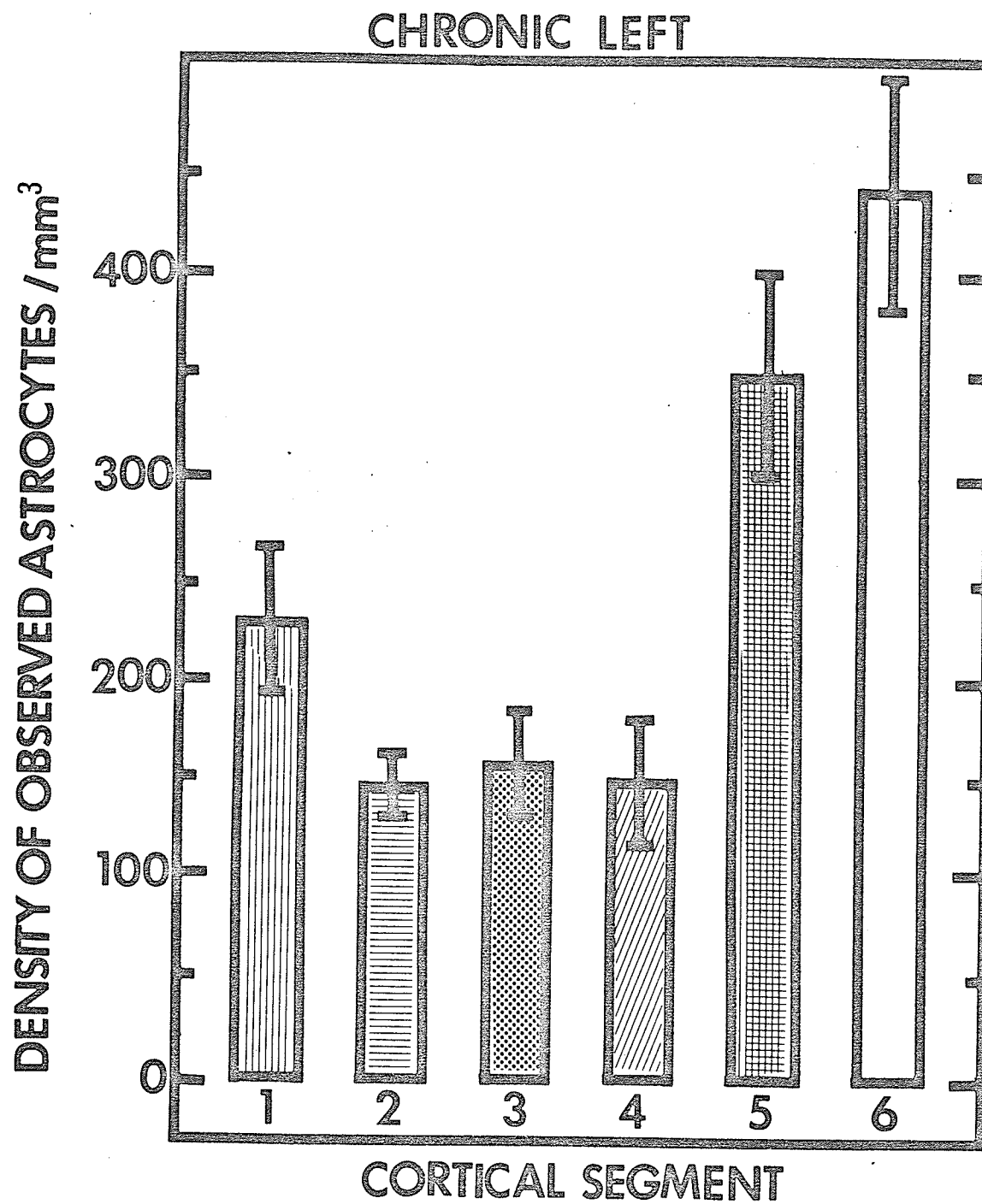


Figure 21. Mean astrocytic population densities in 3 chronically isolated left gyri.

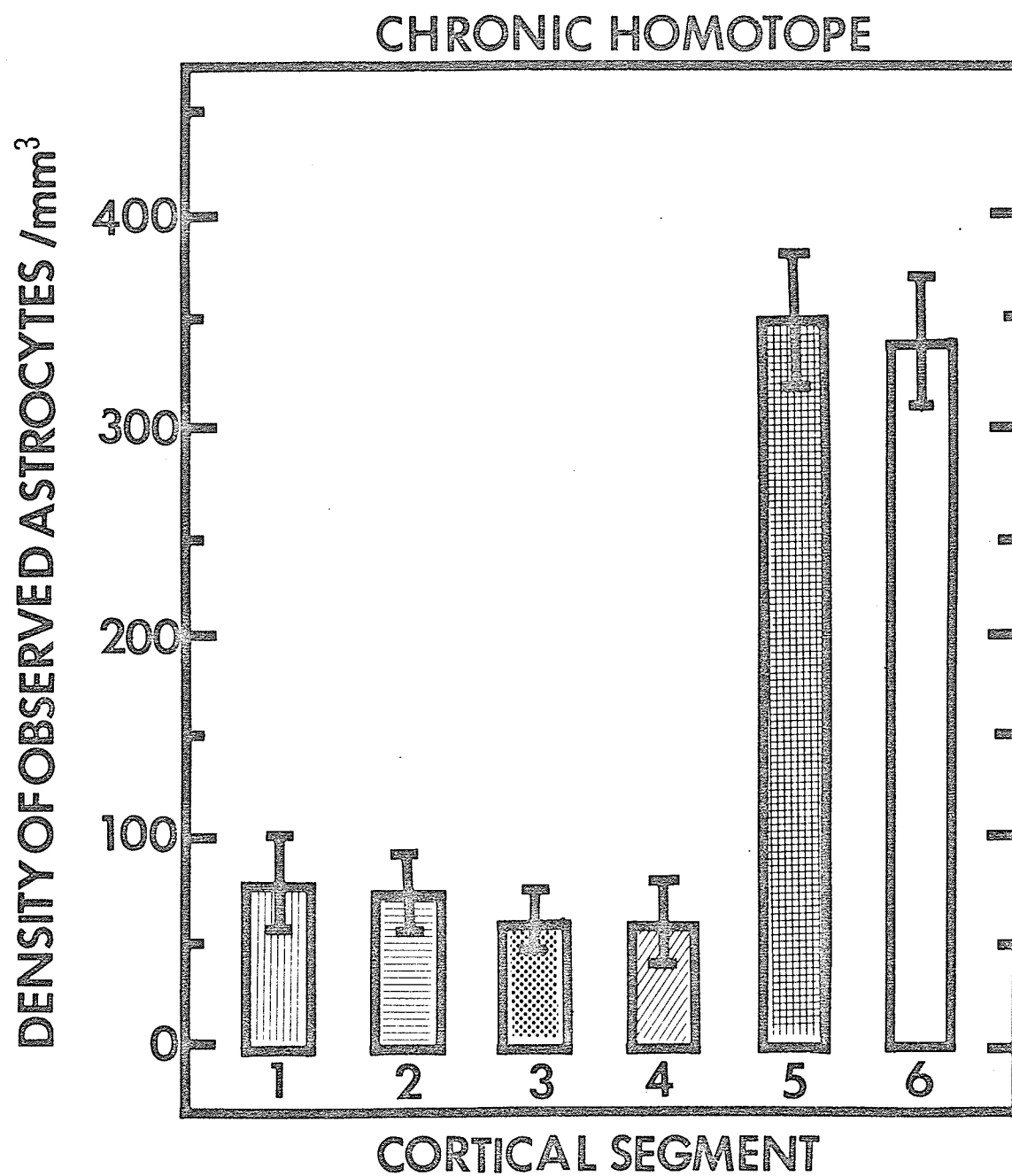


Figure 22. Mean astrocytic population densities in 3 chronic non-operated homotopic gyri.

The data from 11 (eleven) non-operated cortices were grouped, i.e. 4 intact left gyri, 4 intact homotopic gyri and 3 chronic homotopic gyri, and the astrocytic population density was calculated at every "2" mm interval. The results of these calculations are listed in Table 4 and are shown in 3 dimensional histogram form in Fig. 23. At each "2" mm interval the density in the upper cortical half is significantly less when compared to the lower cortical half ($P \leq 0.001$). The mean astrocytic population density for all cortical segments in all the non-operated cortices was calculated and is shown in histogram form in Fig. 24 and as a representative coronal section in Fig. 25.

TABLE 1V

Mean astrocytic population densities in 11 non-operated gyri

Distance from Ant. End <i>IN MM</i>	Cortical Segments					
	1	2	3	4	5	6
0	114	161	127	94	356	296
2	157	141	165	142	311	363
4	79	90	79	42	307	353
5	78	85	103	128	375	402
6	61	75	85	62	363	353
8	86	83	89	51	406	389
10	97	84	95	34	395	389

GROUPED NON-OPERATED CORTICES

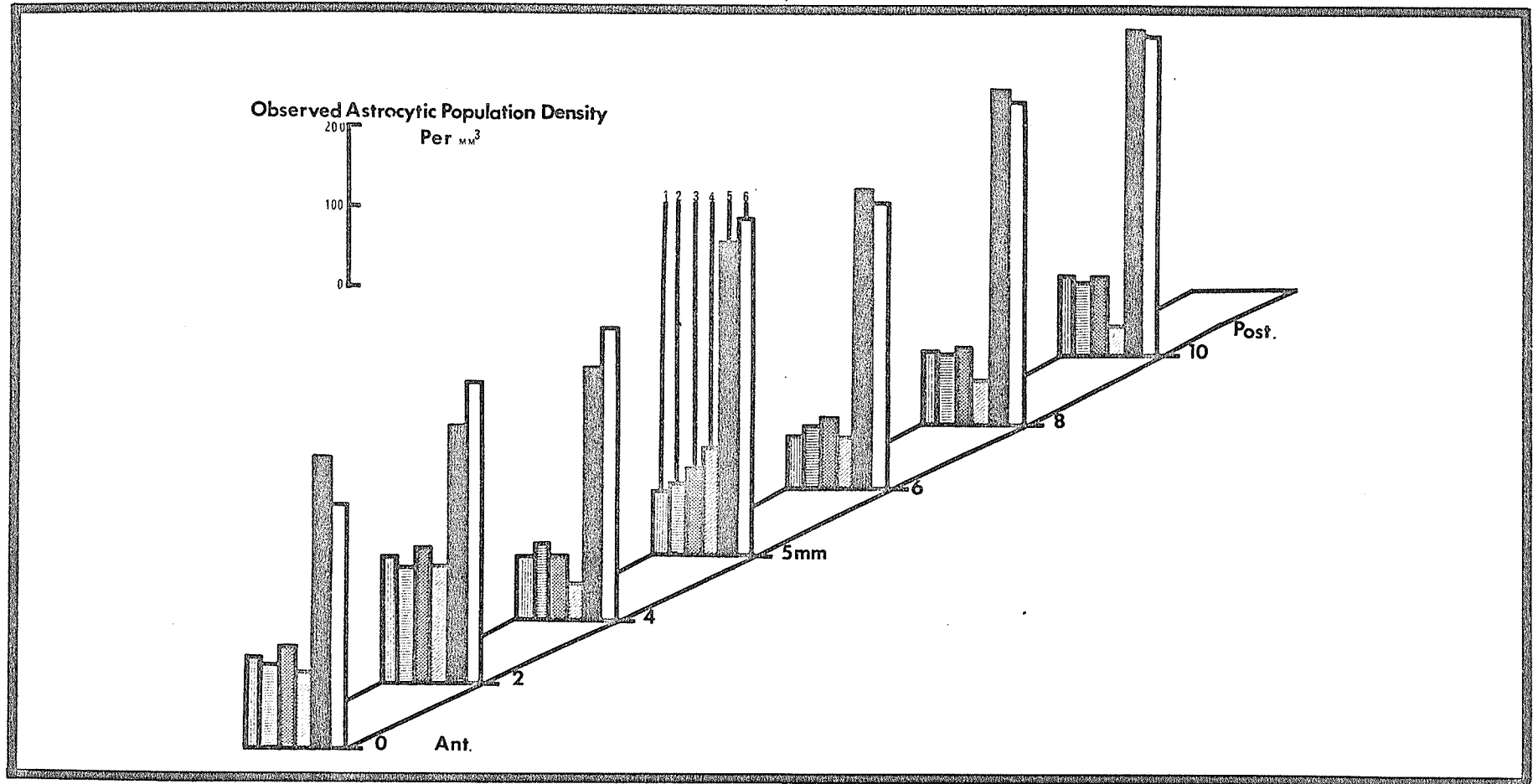


Figure 23. Astrocytic population densities calculated from 11 non-operated cortices i.e. 4 intact, 4 intact homotopes, 3 chronic homotopes.

1	2	3	4
$\bar{X}$ = 117.4	$\bar{X}$ = 116	$\bar{X}$ = 123.47	$\bar{X}$ = 98.76
S = 87.27	S = 62.6	S = 68.5	S = 77.99
SE = 19.04	SE = 13.57	SE = 14.94	SE = 17.02
6		5	
$\bar{X}$ = 328.61		$\bar{X}$ = 304.85	
S = 102.07		S = 99.05	
SE = 22.27		SE = 21.61	

Figure 24. Mean astrocytic population densities
calculated from all non-operated cortices.

$\bar{X}$ = Mean Astrocytic Density.

S = Standard Deviation.

SE = Standard Error.

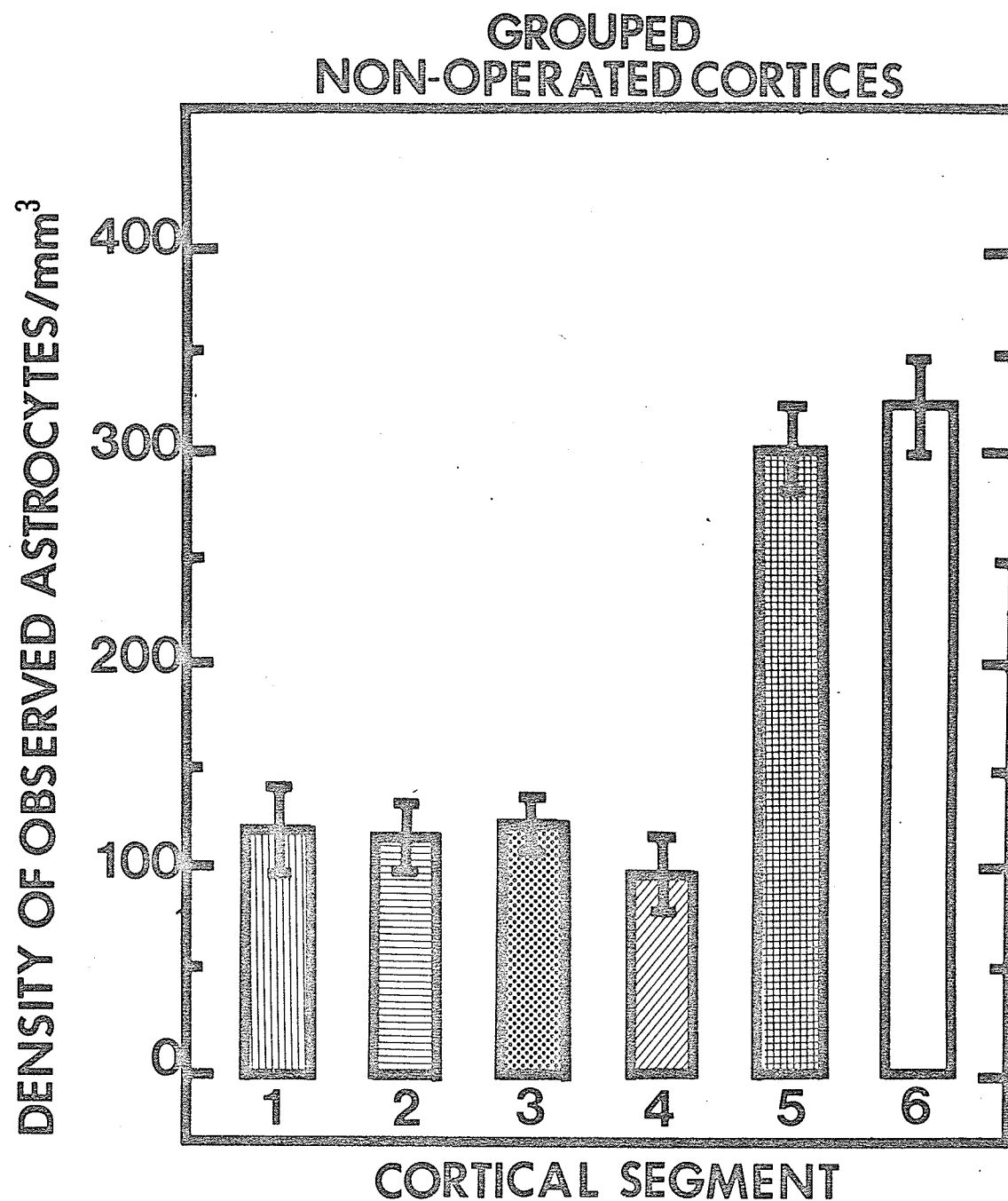


Figure 25. Mean astrocytic population densities in 11 non-operated supra-sylvian gyri.

DISCUSSION

In this investigation a study was made of the effects which chronic and acute isolation of cat cerebral cortex would have on the astrocytic population density. The results from this histological study suggest certain functional properties to cortical protoplasmic astrocytes which will be discussed in a later part of this thesis section.

MORPHOLOGY OF CORTICAL ASTROCYTES.

The identification of astrocytes in the cerebral cortex was based entirely on the morphology and distribution of their cellular processes. All of the cortical astrocytes observed in this study were of the protoplasmic type. In a few instances plasmatofibrous astrocytes were observed at the junction of grey and white matter. These specific astrocytes as well as cells which were impossible to identify were not counted. This method helped to ensure that only positively identified protoplasmic astrocytes were enumerated. Protoplasmic astrocytes are known to form intimate associations with both neurons and blood vessels by means of the terminal portion of their processes. Several observations of this type were found in this study (Plates IV and V). It is, therefore, concluded that only protoplasmic astrocytes were enumerated in this study and are the only type of astrocyte to be found in the cortex of the supra-sylvian gyrus.

A few areas in the cerebral cortex were sometimes found to be devoid of stained cells although blood-capillaries were clearly observed to be present. This problem appeared to be one of tissue-stain refractoriness and is therefore suggested as a future histochemical study of the cerebral cortex.

Histological methods which involve modifications of the classical Golgi technique may possibly involve a combination of physical and biochemical reactions. The cytological architecture of nervous tissue is preserved with the use of a good chemical fixative. In this study, Ramon-Moliner's (1957) technique always showed a uniform impregnation pattern of astrocytes against a yellowish transparent back ground. Sholl (1956) reported that in preparations involving modifications of the Golgi technique, the staining characteristics of the cells are unaffected by lesions or wounds. This would seem to suggest that any observable cyto-morphological changes in astrocytes following brain injury or exogenous electrical stimulation of the brain would be the result of biophysical and biochemical alteration occurring within the cell and therefore not be attributable to the staining procedures used in this study.

BIOCHEMICAL CHANGES IN ACUTELY ISOLATED CORTEX.

Cortical tissues which were subjected to both electrical stimulation and acute isolation appear to affect the biochemical properties of neuronal cells. It is plausible to suggest that neuroglial cells are also involved in these biochemical changes. Any change in the biochemical properties of cortical tissue can be attributed, in part, to intermediate stages of cell metabolism following anoxia, eg, hypoglycaemia. Krass et al (1968) reported that following neuronal isolation there is an increase in glucose metabolism within the cortical slab. This increase in glucose metabolism is also observed to "parallel" for a few weeks a highly significant reduction in the inherent electrical activity within the slab (Vazquez, Krip and Pinsky, 1969). These findings would seem to indicate that changes in the intermediate stages of glucose metabolism would also be found in cortical astrocytes.

Changes in the biochemistry of acutely isolated slabs could not have resulted from a depletion or reduction of blood supply. Both macroscopic and microscopic observations showed that blood vessels in the pial- surface were apparently normal; also, no instances of necrosis in the protected areas in the upper cortical half were noted to have occurred. Histological sections of acute slabs used in this study showed a high number of blood-capillaries. Pinsky (1961) reported that the blood supply in isolated slabs is adequate to help

maintain electrical after-discharges even after long-term isolation. Reiffenstein (1964) concluded from his histological studies that it was even possible to consider that the blood-supply had increased rather than decreased in the isolated slabs. With an adequate supply of blood, it is difficult to explain the reduction in astrocytic population density except from a physical and biochemical aspect. It is, therefore, suggested that the reduction or "fall-off" in the density of observable astrocytic cells in acutely isolated cortex represents a form of chemical and/ or physical refractoriness of astrocytes to the staining procedures and may not necessarily be indicative of any real fall-off in astrocytic population density.

Immediately following isolation, the cortical tissue is known to be severely traumatized at the posterior end of the supra-sylvian gyrus where the aspiration hole had been made. The posterior regions of both left and right acute slabs showed tendencies towards an increase in the astrocytic population density. It is tempting to suggest that although an immediate response of astrocytosis was to be expected the staining refractoriness resulted in a non-significant increase in the astrocytic population density observed in these two areas.

CHRONICALLY ISOLATED CORTEX.

Axotomy is the consequence of neuronal isolation resulting from the surgery of the undercut and the two side

cuts. Axotomy is also accompanied by an immediate and progressive degeneration of neurons in the cerebral cortex (Cajal 1928). It has been well documented that neurons whose axons are cut proximal to the first axonal collateral will undergo immediate degeneration. In the present study neurons which were present in the lower cortical half as well as those which were adjacent to the side cuts could be expected most affected by axotomy. Neuronal Isolation is known also to result in a significant loss of dendritic density in the protected and non-protected areas in the upper cortical half (Weisman et al 1967). The greatest fall off in dendritic density was found to occur in the upper cortical half in chronically isolated cortex. The side cuts would also sever dendritic processes leaving several distal stumps which would be expected to undergo degeneration. In this study it was found that in chronic slabs, there was a significant increase in astrocytic population density in the protected and non-protected areas of the upper cortical half when compared to non-operated cortex. The population density increase was found to be uniform in all upper chronic segments.

A fundamental question which must now be asked is: "Can protoplasmic astrocytes in the cerebral cortex divide?"

Earlier studies by several authoritative investigators have attempted to ascertain whether neuroglia, taken from mammalian post-natal brains and not influenced by neoplasm were capable of cell division. Nakai and Okamoto (1963) have

observed amitotic cell division of astrocytes to occur in tissue culture. Lumsden (1963) reported similar results for in vitro studies of neoplastic neuroglia. He also observed binucleate glial cells as being indicative of amitotic nuclear division without the accompaniment of cytokinesis. Fibrous astrocytes have been observed to divide mitotically in normal and in degenerating white matter (Mori and Leblond(1969); Vaughn et al (1970)).

Vaughn and Peters in 1967 and Vaughn in 1968 advanced the hypothesis that neuroglia, which they renamed multipotential glia may differentiate into astrocytes or into oligodendrocytes. Miquel and Haymaker (1965) reported that numerous mitotic figures were seen in cerebral cortical glial cells following X irradiation. They reported also that in each of the dividing cells the cytoplasm was rich in glycogen. Aleksandrovskaya and Kholodov (1966), following an E.E.G. study with electrodes in-planted in the bone of cats and rabbits, reported that astrocytes in the cerebral cortex had divided by amitosis. Indirect evidence is now advanced in this section of the thesis to support the concept that protoplasmic astrocytes in the mammalian cerebral cortex divide in response to an increased physiological demand.

Several mechanisms are suggested which could possibly account for the increase in astrocytic population density in

the upper cortical halves of chronic slabs. First, the physiological influences which determined the extent of astrocytosis is uniformly distributed throughout the width of the slab and not necessarily confined to the immediate areas of the two side cuts. The significant loss of dendritic density in the entire width of the upper cortical half would seem to suggest that spaces are formed. It is believed by many authorities that neuroglial cells function as the reticular tissues of the nervous system. It is, therefore, tempting to suggest that a loss of dendritic density in the cerebral cortex is followed by a corresponding increase of astrocytic population density as a means of filling the new spaces.

Secondly, recent studies by Vaughn and Pearse (1970) have shown that fibrous astrocytes become phagocytic in rat optic nerves in which Wallerian degeneration of myelin is present. They have suggested also that pathology in the C.N.S. which is the result of surgical trauma induces pluri-potential requirements for phagocytosis and cellular proliferation.

Vaughn and Pearse (1970) inferred also that the degree to which several different neuroglia may be recruited for phagocytosis is related to the severity of neuronal degeneration within the immediate area. In this study it is suggested that these same influences are uniformly distributed

in all segments of the upper cortical half throughout the length of chronic slabs. This uniformity is evidenced by the persistence of similar population density profiles which are shown in Fig. 17.

Thirdly, Campbell (1939), has shown that the density of blood capillaries per cubic millimeter of cerebral cortex in the upper cortical half is significantly less when compared to the lower cortical half within the same gyrus. Although pial blood vessels over the slab were not injured during the surgical procedures it is reasonable to assume that as a result of the two side cuts there would be a tendency towards a reduction in the volume of blood flow in the upper cortical half when compared to the pre-operated cortex. The reduced blood volume would also parallel a decrease in the nutritional supply to neuronal elements by way of the protoplasmic astrocytic pathway. An increase in astrocytic density would therefore be an effective method of compensation for the reduced blood flow. Since the greatest fall-off in neuron population density occurred in the lower cortical half the physiological demand for nutrition supplying astrocytes is correspondingly less.

Astrocytic cells occupy approximately 5.23% of the interneuronal spaces in the gray matter (Ramon-Moliner 1961). There have been several reports that extra cellular spaces in the central nervous system are non-existent (Wyckoff and Young 1956; Luse 1956; Gerschenfeld et al (1959). De Robertis

et al (1961) reported that the maximum size of any inter-cellular space in the central nervous system, other than synaptic clefts, would not be more than $200 \text{ \AA}$. Histological studies in the cerebral cortex have shown that astrocytes form the principal pathway for metabolites and water between capillaries and nerve cells (De Robertis and Gerchenfeld 1961). This concept has led some investigators to attribute astrocytes with the Blood-Brain Barrier Mechanism (Tschirgi 1958). Stab wounds in the brain are followed by a significant increase in glycogen accumulation within the traumatized area Friede, 1954; Shimizu and Hansuro, 1958; Estable - Puig et al (1963). Miquel and Haymaker (1965) reported that cortical lesions which were the result of X-irradiation gave rise to glial cells rich in glycogen. Krass et al (1968) reported that increased glycolysis in chronically isolated cerebral cortex in cats persisted for a period of two weeks after the initial isolation. The increased presence of glycogen in these traumatized areas of the brain would seem to indicate that there is a requirement for an increase in energy for the repair and healing mechanism. Since glucose metabolism is associated with astrocytes (Lumsden 1968) the increase in astrocytic population density would parallel the increase in cortical glucose content.

It is, therefore, suggested that an increase in the density of protoplasmic astrocytes with branching processes would have the effect of increasing the number of nutritional

pathways in response to an increased physiological demand for new astrocytes.

Lumsden (1968) has postulated the view that

" glia might be bipotential in vivo in the sense that oligodendrocytes might become converted into astrocytes although neuropathological evidence makes it improbable that the reverse change (astrocyte to oligodendrocyte) can occur."

Astrocytosis, a reaction to trauma, was postulated also to exhibit limited migration (ibid). The mean depth of the cortex in chronic slabs was about 1300 microns. Astrocytes which would be situated 850 microns below the pial surface, need migrate a maximum of 400 microns to be enumerated in the upper cortical half. It is suggested also that as a result of bilateral shrinkage and compression in the lower cortical half of chronic slabs (Weisman 1969) the total volume of empty spaces left by degenerating neuronal somata is considerably reduced in that specific area.

It is, therefore postulated that the significant increase in astrocytic population density in chronically isolated cerebral cortex is a consequence of several physiological mechanisms which resulted in astrocytosis.

SUMMARY

- (1) Neuronally isolated cortical slabs were prepared in such a way that its blood supply was the only remaining connection with the rest of the brain. The slabs were cut within the margins of the suprasylvian gyrus.
- (2) Histological sections showed that in acutely and chronically isolated slabs there were no cellular connections from neurons or neuroglial cells with surrounding intact tissues.
- (3) Quantitative estimation of astrocytic population densities were carried out on histological preparations stained according to Ramon-Moliner's (1957) technique. Results from these studies showed:
 - (a) In all non-operated and isolated cortices, the astrocytic population densities were significantly less in the upper cortical half when compared to the lower cortical half.
 - (b) In acutely isolated cortex there was a significant decrease in astrocytic population density in the lower cortical segments when compared to similar areas in intact and chronic cortex. This decrease is believed to be associated with the change in intermediate cellular metabolism.
 - (c) In chronically isolated slabs, it was found that there was a localized increase in astrocytic population density which was confined to the upper

cortical half.

This increase coincided with those chronic segments where a significant "fall-off" of dendritic density has been reported earlier. The lower cortical segments, 5 and 6, showed no significant increase in astrocytic population densities when compared to non-operative gyri except at the region nearest to the posterior drainage hole. The increase in astrocytic population densities in the chronically isolated slabs seemed to be a consequence of the experimental procedures and new functional conditions which occurred in the isolated cortex. Several citations in this thesis are in agreement with the finding that astrocytes can undergo multiplication.

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