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ALTERNATE CEREBELLAR CIRCUITRY - THE MORPHOLOGY OF
THE CAUDAL CEREBELLAR LOBE OF WEAKLY ELECTRIC FISH

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DEDICATION

The work represented by this manuscript was possible through the contributions of many, but none so important as those consistently given to me by my wife, Charlene, throughout the course of my study. To her patience, love and support I dedicate this thesis.

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ABSTRACT

A light and electron microscopic investigation of the caudal cerebellar lobe of weakly electric fish reveals a basic morphology similar to that of most vertebrate cerebellae. The morphologies of the Purkinje, stellate, Golgi and granule cells parallel descriptions of similar neurons found in other teleost as well as higher vertebrate species. Notably absent is the basket cell. As in most teleosts, the cerebellum lacks any deep cerebellar nuclei. A large neuron, the eurydendroid cell, displays many of the morphological characteristics and synaptological relations shown by efferent neurons of higher vertebrate cerebella but is anomalously positioned in the cerebellar cortex of the weakly electric fish.

A curious feature of the caudal lobe morphology and a somewhat aberrant distinction from the classic cerebellar plan is the "pars medialis" granule cell layer of the caudal lobe. Associated with this and extending into the molecular layer are displaced or "ectopic" granule cells. Residing upon a pial surface, the caudal lobe "pars medialis" is believed to be the undescended neurons of the external germinal layer.

INTRODUCTION

I MORPHOLOGICAL FEATURES OF THE MAMMALIAN CEREBELLUM

A. Cerebellar Cortex

The cerebellum has long been considered an integrator where converging sensory information is processed and returned to effect a smooth motor response (Eccles et al., 1967; Eccles, 1977; Ito, 1980). Confronted with the mysteries of how the cerebellum acts to coordinate these sensory modalities, investigators have explored the morphology of the afferent and efferent pathways in conjunction with its intrinsic circuitry. The mammalian cerebellum is a simple laminar structure composed of a few distinct layers (Larsell, 1952). A superficial molecular layer resides just under the outer pial covering. Beneath this stratum is a band of densely packed neurons referred to as the granule cell layer. Interposed between these two laminae is a region composed of a monolayer of large neurons called Purkinje cells, and referred to as the Purkinje cell layer. The molecular, Purkinje cell, and granule cell layers combined, constitute the cerebellar cortex (Eccles et al., 1967). The remaining cerebellar tissue is referred to as the "white matter" (Larsell, 1952). Embedded deep within this white matter and in juxtaposition to the brainstem is a bilaterally symmetrical nuclear mass called the deep cerebellar nuclei (Korneliussen, 1968; Korneliussen, 1969; Chan-Palay, 1977). Five major neuronal types are found within the mammalian cerebellar cortex - Purkinje, Golgi, granule, stellate and basket cells. Purkinje and granule cells, because of their size and the unique form of their dendritic arborizations, are the most distinguishable.

PURKINJE NEURONS have a densely branching tree of spiny dendrites, oriented in planar fashion, that arise from a single apical trunk of a large soma to arborize in the molecular layer of the cerebellar cortex (Cajal, 1955; Ariens-Kappers et al., 1967). The fine structure of this neuron is as distinctive as its characteristic morphology viewed in light microscopy. A dense cytoplasmic matrix is filled with rough endoplasmic reticulum (RER), mitochondria, Golgi apparatus and numerous free ribosomal material that imparts considerable density to the matrix (Larramendi, 1969). These organelles are arranged around a large central nucleus in a "whorl-like" fashion (Uchizono, 1969; Palay and Chan-Palay, 1974). A thin glial sheath that surrounds the cell is typical of the Purkinje neuron observed in the Electron Microscope (EM). Occasionally, terminals believed to be from recurrent Purkinje collaterals have been observed contacting the somata of these large neurons (Uchizono, 1969). The dendrites of the Purkinje (P) cell are distinguishable from other processes principally by the numerous stubby spines that are filled with cisternal elements (Larramendi, 1969; Palay and Chan-Palay, 1974; Schiebel et al., 1981).

GRANULE CELLS were studied quite extensively by Cajal in his pioneer work at the turn of the century (Cajal, 1955). These small neurons are compactly arranged principally within the granule cell lamina of the mammalian cerebellar cortex. From a spherical soma fostering 3 - 5 short radiating dendrites, arises a thin axon that

traverses the granule cell layer and extends into the molecular layer (Braitenberg and Atwood, 1958). Here these axons bifurcate and, together with many other granule cell axons, form what has classically been described as parallel fibers (Fox et al., 1969). Ultrastructurally, a large nucleus, devoid of any nucleolus, dominates the granule cell perikaryon. A few mitochondria, Golgi apparatus and ribosomes constitute the organelles of the cytoplasmic matrix (Palay and Chan-Palay, 1974). Often, granule cells are observed to be clustered in groups of 6 - 9 cell bodies but their most striking feature is the involvement of their short dendrites with MOSSY FIBER terminal enlargements forming what has long been described as the glomerulus (Gray, 1961; Kaiserman-Abramof and Palay, 1969). These mossy fibers form an interdigitated rosette around which synapse the club-shaped varicosities of the granule cell. Superimposed upon the glomeruli is the axonal terminal of another granule cell layer neuron, the Golgi cell (Hamori and Szentagothai, 1966) which will be described below. Between the "en passant" mossy fiber terminals and granule cell dendrites of the mammalian cerebellum are found two distinctly different synaptic zones. One is the conventional synaptic zone while the other is referred to as a "gap junction" (Sotelo and Llinas, 1972). Two classes of mossy fibers have been described in the granule cell layer of the mammalian cerebellar cortex. Referring to the degree of compaction of their vesicle populations and, presumably, implying that their extracerebellar origins are varied, these terminals have

been defined as mossy fiber c and d (compact and dispersed, respectively) (Palay and Chan-Palay, 1974). Recent evidence also suggests that recurrent collaterals of projective nucleofugal fibers are mossy in nature and terminate within the granule cell layer of the rat cerebellum (Hamori et al., 1981). The granule cell acts as an interneuron, relaying information transmitted by mossy fibers to cells whose dendrites permeate the molecular layer (Eccles et al., 1966; Hamori and Szentagothai, 1966). The most prominent example of this is seen between the parallel fiber terminals and the P cell dendrites in the intact cerebellar cortex (Uchizono, 1969; Larramendi, 1969) as well as those grown in organotypic cultures (Aggerwal and Hendelman, 1980).

CLIMBING FIBERS are clearly distinguishable from the other major terminals of the molecular layer, namely parallel fiber boutons. In Golgi-impregnated material climbing fibers, as the name suggests, appear to form a profusely twining web around the dendrites of the molecular layer (Cajal, 1955). In the EM, they are most prominently viewed as they terminate on the lateral surface of 2 - 3 Purkinje cell spines (Larramendi, 1969). Parallel fibers, on the other hand, rarely contact more than one spine, usually on its distal end. Numerous vesicles, (including some dense core varieties) as well as mitochondria make up the internal structure of the climbing fiber terminal. Usually, only a few clear vesicles are found in the parallel fiber terminal (Palay and Chan-Palay, 1974). In the mammalian cerebellum, climbing fibers appear to originate from one

principal source, the inferior olivary nucleus (Groenewegen et al., 1979; Courville et al., 1980). From the inferior olive, the climbing fiber enters the cerebellum, passing collaterals to the deep nuclei and continues only to terminate in the molecular layer of the cerebellar cortex. Some evidence has been given for the presence of climbing fiber terminals within the granule cell layer (Palay and Chan-Palay, 1974).

INTERNEURONS constitute the remainder of the cells in the mammalian cerebellar cortex. The two classes of GOLGI NEURONS residing in the granule cell layer are virtually indistinguishable from each other, morphologically, except on the basis of somatal size (Cajal, 1955). A smaller variety is found deep within the granule cell layer and its dendrites ramify mainly within this lamina. A larger Golgi cell is positioned adjacent to the Purkinje cell layer but the arborization of their dendritic processes is primarily within the molecular layer (Palkovits et al., 1971). From the round Golgi perikaryon radiate thin, sinuous dendrites, devoid of any spines. These processes branch profusely assuming a spherical, rather than planar, orientation around the cell body. The axons arise from the soma or proximal dendritic trunks to form a considerable plexus in the vicinity of the cell body (Palay and Chan-Palay, 1974). Ultrastructurally the Golgi cell is distinguishable by its intermediate size between the larger Purkinje neurons and the small granule cells that surround it. The perikaryon has a lobulated nucleus with a prominent nucleolus. Golgi apparatus,

mitochondria and Nissl bodies surround the nucleus. Numerous free ribosomes impart a density to the Golgi cell cytoplasm, to a lesser extent but in much the same fashion as the Purkinje cytoplasm (Palay and Chan-Palay, 1974). The surface of the Golgi cell body is closely apposed to the numerous adjacent granule cells. Occasionally, a number of synaptic contacts can be seen upon the Golgi perikaryal surface. Axially oriented microtubules and mitochondria characterize the thin dendrites of the Golgi neuron. However, the most striking part of this cell's ultrastructure is seen with the axon terminals and their involvement with the glomerulus. Flattened boutons are often seen contacting the "preterminal intraglomerular parts of the granule cell dendrites as well as their terminal spheroid protrusions" (Hamori and Szentagothai, 1966). It is believed that the Golgi neuron is inhibitory in nature (Eccles et al., 1966).

STELLATE CELLS and BASKET CELLS are interneurons found within the molecular layer of the mammalian cerebellar cortex. The basket cell is usually found in the lower one third of this lamina in close association with the Purkinje cells (Cajal, 1955). From a pyramidal-shaped soma, spiny branched dendrites course through the molecular layer. Entwining the cell body and proximal dendrites of the Purkinje neuron is the complex axon plexus of the basket cell (Fox et al., 1967). Stellate cells are a class of polymorphous neurons that are found in the upper two thirds of the molecular lamina. Their contorted dendritic ramifications radiate in all directions

from an oval to cigar-shaped nucleus (Lemkey-Johnston and Larramendi, 1968). This contrasts the relatively straight course assumed by the dendrites of the basket cells (Cajal, 1955). The target of the stellate axon terminals, again appears to be the Purkinje cell (Chan-Palay and Palay, 1972). In their extensive treatise on the morphology of the rat cerebellar cortex, Palay and Chan-Palay (1974) describe another neuron called the LUGARO CELL. Some Lugaro somata are oriented horizontally within a ganglionic or plexiform region just below the Purkinje cell layer. Dendritic processes often run bipolarly, in a horizontal plane, to ramify extensively within the molecular, and granule cell, layers. In some, the dendrites may extend some distance running completely through the granule cell lamina (O'Leary et al., 1968). Ultrastructurally, a random orientation of small mitochondria, Golgi apparatus and a few ribosomes characterize a cytoplasm that is dominated by a large nucleus. Somatic contacts by Purkinje axon recurrent collaterals have been seen on the surface of the Lugaro perikaryon and proximal dendrites (Palay and Chan-Palay, 1974). The fleshy dendrites of this neuron have the occasional stubby spine and some neurofilamentous material is axially positioned within the processes. The axons of the Lugaro cell are directed towards the molecular layer of the cerebellar cortex (O'Leary et al., 1968).

B. Deep Cerebellar Nuclei

An important feature of the mammalian cerebellum is the presence of the deep nuclei embedded within the white matter. Believed to be the main efferent component of the cerebellum, the cells of the deep nuclei receive afferent collateral fibers as well as Purkinje axon terminals originating from the cerebellar cortex (Groenewegen et al., 1979; Dietrichs, 1981). In mammals two main groups of cells are found throughout the lateral or dentate nucleus (Chan-Palay, 1977). There is a larger type that remains peripheral and a smaller variety that is often identified deep within the nucleus itself. Axons of a few of the smaller neurons course some distance through the lateral nucleus forming what is referred to as the dentato-olivary connections that are efferent in nature (Chan-Palay, 1977). Many of the large neurons of the deep nuclei have bipolar dendritic trees that emanate from an oval or elliptical cell body. Occasionally stubby spines are found on the dendrites of these neurons as well as on the surface of the perikaryon itself. Along the lengths of the dendritic ramifications, irregular expansions that have been described as "frond-like" excrescences, are prominently seen (Chan-Palay, 1977). The fine structure of the large neurons in the lateral nucleus of the rat cerebellum depicts a large nucleus around which are found dispersed ribosomal material, numerous small mitochondria, large Nissl bodies and a well developed Golgi apparatus. Upon the perikaryal surface are a number of synaptic contacts. Among the longitudinally running mitochondria and microtubules within the dendritic processes of the large cells are prominent fascicles of

neurofilaments running axially (Chan-Palay, 1977).

II COMPARATIVE ASPECTS OF VERTEBRATE CEREBELLAR MORPHOLOGY, INCLUDING TELEOSTS

The basic structure characterizing the mammalian cerebellum appears to be well conserved throughout the vertebrate phyla (Cajal, 1955; Larsell, 1967). Some variations do occur, however, and are particularly visible in the teleosts (Larsell, 1967; Llinas, 1969). The deep cerebellar nuclei in most fish are absent. Instead, a large neuron, which will be described in detail later, appears to serve the role of the efferent pathway (Nieuwenhuys et al., 1974; Finger, 1978b). The laminar cortex of the cerebellum of mammals and that of lower vertebrates has been found to be quite similar (Hillman, 1969; Llinas, 1969). In teleosts, there is a granule cell layer, a molecular layer and an intermediate, Purkinje cell or, plexiform region (Pouwels, 1978b). Medially and mid-caudally the cerebellum of some teleosts contains a group of granule cells that have been referred to as 'pars medialis' (Larsell, 1967; Maler et al., 1974). Most of the neuronal elements found within the cortex of the higher vertebrate cerebellum are present in species of the lower phyla. Notably absent from the teleost group, however, is the basket cell (Cajal, 1955; Larsell, 1967). P cells are among the largest neurons of the teleost cerebellum. Larsell (1967) describes this cell from primitive vertebrates through the bird cerebellum. Its form appears to be clearly distinguishable from the other neuronal elements by

nature of the prolific spine growth on their dendritic branches (Ariens-Kappers et al., 1967). The arborizations of the more primitive P neurons are mostly planar within the molecular strata and arise as a single stock from the apical pole of the soma. The one noticeable difference between Purkinje dendrites of higher and lower vertebrates is the diminished branching in the latter, rarely beyond the tertiary level in most teleosts (Cajal, 1955; Larsell, 1967; Llinas, 1969). The P cell ultrastructure of amphibians (Sotelo, 1969) as well as teleosts (Nieuwenhuys and Nicholson, 1969b) bears striking resemblance to those found in mammals (Uchizono, 1969). The perikaryon is almost completely enveloped in a thin glial sheath. Although the nuclei rarely exhibit lobulation or infoldings typical of higher vertebrates, they centre an array of cytoplasmic organelles, such as mitochondria, Golgi apparatus, RER, and free ribosomes within a dense matrix in similar "whorl-like" fashion. The Purkinje cell dendrites are prominently visible in the molecular layer because of the preponderance of short spines that completely cover their processes. Within the spines found on some teleost Purkinje neurons, is considerable cisternal material (Nieuwenhuys and Nicholson, 1969b).

Throughout the vertebrate phyla, granule cells show remarkable conservation in their morphology. Their relationship with the afferent mossy fiber terminals assumes the classical glomerular form including the complex interdigitations between the club-shaped granule cell claw and the mossy rosette (Hillman, 1969; Pouwels, 1978c).

Terminating upon the exterior of the glomeruli are the Golgi cell boutons, flattened and containing a pleomorphic vesicle population. As found in the mammalian cerebellar cortex, axons of granule cells of lower vertebrate classes traverse the granule cell layer and bifurcate within the molecular layer forming an extensive network of parallel fibers (Larsell, 1967). Some evidence has been given for a few teleost granule cells that do not bifurcate in the developing cerebellum (Pouwels, 1978b). Ultrastructurally, the classical parallel fiber - Purkinje spine contact that predominates in the mammalian cortex, shares a similar clarity of distinction in amphibians (Sotelo, 1969) and teleosts (Nieuwenhuys et al., 1974). Even in these lower vertebrates, the characteristic features distinguishing parallel fibers and climbing fibers are clearly established. Where the granule cell boutons are small and round containing but a few small clear vesicles, the climbing fiber terminals are three to four times the size, containing mitochondria as well as many vesicles, including a number of larger dense core varieties (Kaiserman-Abramof and Palay, 1969; Hillman, 1969). The climbing fiber terminals may be found synapsing with the lateral surfaces of 2 - 3 Purkinje spines as well as the main dendritic trunk; while parallel fibers terminate on the distal portion of only one stubby spine. Even at the light microscopic level, the climbing fiber of the teleost cerebellum, as the name suggests, climbs all over dendrites found in the molecular layer (Finger, 1978a).

Interneurons of the cerebellar cortex of lower vertebrates, particularly teleosts, are quantitatively attenuated as compared to mammals. As previously mentioned, the basket cell does not exist in most fish species. Stellate interneurons, though few in number are similar to those found in the mammalian cerebellar cortex (Larsell, 1967; Llinas, 1969; Pouwels, 1978b). Throughout the vertebrate phyla Golgi cells can be found in the granule cell layer (Cajal, 1955; Larsell, 1967; Llinas, 1969). Two groups, distinguished chiefly by cell body size, are evident in amphibians (Hillman, 1969) and teleosts (Nieuwenhuys et al., 1974):

Some Golgi cells previously described in the literature may have been misclassified. Larsell (1967) described a Golgi neuron lying close to the plexiform layer in some teleosts that has a morphology similar to descriptions given for deep cerebellar nuclear cells. Other cells have been described that have similar positions and forms. Cajal (1955) described a "cellule fusiforme horizontale" that was positioned in the granule cell lamina, subjacent to the Purkinje cell layer of teleosts. Other neurons with similar morphologies have been described in the mammalian cerebellar cortex and identified as a Lugaro cell (O'Leary et al., 1968). More recently, the giant cells of the mormyrid (teleost) cerebellum (Nieuwenhuys and Nicholson, 1969a) and the eurydendroid neurons of other teleost cerebella (Pouwels, 1978b; Finger, 1978b) share many of the same characteristic features. It is believed that the giant cell of the mormyrid cerebellum and the eurydendroid cells found in trout

(Pouwels, 1978c) and catfish (Finger, 1978b) are all efferent in nature, which parallels the role of the deep cerebellar nuclear cells.

Nieuwenhuys and Nicholson (1969a,b) point out that the giant cells of the mormyrid cerebellum have a distinctly different morphology than adjacent Purkinje neurons. The smooth slightly beaded dendrites of these neurons sharply contrast the spinous Purkinje dendrites. By its very name, the eurydendroid cell (eury-wide; dendroid-branching) parallels the description given by Catois, cited in Cajal (1955), of a neuron whose dendritic field occupied one-third of the cerebellum. By the fact that most teleosts lack deep cerebellar nuclei, this efferent neuron has attracted some interest (Nieuwenhuys et al., 1974; Pouwels, 1968b; Finger, 1978b). The ultrastructural characteristics of this cell have received little attention. In the trout it has been shown that a few small mitochondria, RER, and some ribosomal components surround a slightly eccentric nucleus. In the developing trout embryo, axosomatic synapses by P cell recurrent collaterals were common upon the perikaryal surface and proximal dendrites of eurydendroid cells (Pouwels, 1978c). Little mention was made concerning any other synaptological relations between these cells and other components of the teleost cerebellum.

III PERSPECTIVES ON CEREBELLAR DEVELOPMENT

From a developmental point of view the cerebellum, with its few neuronal classes and simple laminar structure, has received considerable attention throughout the vertebrate phyla (Larramendi, 1969; Altman and Bayer, 1978; Pouwels, 1978a,b,c). In examining the histogenesis of the trout cerebellum, Pouwels (1978c) identified Purkinje, granule, stellate, Golgi and eurydendroid cells. Purkinje, eurydendroid and Golgi neurons were found to be derived from the ventricular matrix. A secondary matrix acted as a substrate for smaller neurons, such as granule and stellate cells. Similar observations have been made in the developing mammalian cerebellum. In mice embryos, cells of the deep cerebellar nuclei and Purkinje neurons were found to come from the primitive ependyma (Miale and Sidman, 1961; Larramendi, 1969). Altman and Bayer found that in rats, Golgi neurons also developed from the neuroepithelium rather than from the external germinal layer, from which granule neurons and other small cells are derived (Altman and Bayer, 1978). These authors also noted that the development of the caudal portion of the rat cerebellum, known as the flocculus, appeared to be much slower than the remainder of the cerebellum. This apparently conservative developmental strategy found throughout the vertebrate

phyla parallels a curious migrational phenomena observed for some cerebellar neurons. "Ectopic" or displaced granule cells have been identified within the molecular layer of the normal adult rat cerebellum (Chan-Palay, 1972; Stoughton et al., 1978). These cells have been described as "having a focus that is a conical mound of small neurons with its base flat against the pial surface" (Palay and Chan-Palay, 1974). Synaptological homologues of the glomerular structure involving granule cell dendrites and mossy fiber rosettes, have been identified for these ectopic cells, with the notable absence of Golgi cell terminal interactions (Palay and Chan-Palay, 1974; Stoughton et al., 1978).

IV ARCHICEREBELLUM

One cerebellar region that has received a considerable focus of attention from morphologists and physiologists is the lobe that resides caudal to the posterolateral fissure. Referred to as vestibulocerebellum, flocculonodular lobe and, flocculus in mammals as well as the auricular lobe in amphibians, this area has been generally considered archicerebellum (Llinas, 1969; Bell and Dow, 1967). In teleosts, this region of archicerebellum referred to as the caudal lobe (Maler, 1974; Maler et al., 1976) has been found to be equivalent to parts of the vestibulocerebellum of mammals. Extensive investigation of the vestibulocerebellum or flocculus in higher vertebrates, has

implicated it in moderating a bisensory reflex known as the vestibuloocular reflex (VOR) (Robinson, 1976; Baker and Berthoz, 1977; Bender, 1980). In this system, visual information from the retina is relayed via brainstem nuclei to the cerebellar cortex in the form of climbing fibers (Courville et al., 1980). Vestibular information reaches the same cerebellar lobe either indirectly, through the brainstem vestibular nuclei, or directly from the vestibular apparatus. Both arrive in the form of mossy fibers (Brodal and Hoivik, 1964; Simpson et al., 1974; Walberg, 1975). The efferent pathway affecting the smooth motor response for eye-head coordination usually involves cells of the deep cerebellar nuclei.

The archicerebellum of teleosts generally appears to be a caudolateral expansion of the cerebellum proper. In some teleosts, this caudolateral bulge is quite large occupying almost half of the cerebellar volume (Maler, 1974). It is divided from the remainder of the cerebellar tissue by a deep caudolateral fissure and from the brainstem by a marked sulcus. Cholinesterase staining of the mammalian flocculonodular lobe exclusive to the rest of the cerebellum (Silver, 1967) is also characteristic of the archicerebellum or caudal lobe of the teleosts of the Gymnotiform order (Maler et al., 1981). The archicerebellum of these fish receives an indirect electrosensory and proprioceptive input (Maler, 1974; Maler et al., 1976) as well as some visual information (Bastian, 1976). Its involvement in coordinating the body movements of these fish has been the subject of a number of investigations (Bastian, 1975;

Maler et al., 1976; Bastian, 1976).

V RESEARCH PROPOSAL

In the mammalian cerebellar system, the difficulty in discerning the nature of floccular control over the VOR lies in part, with the circuitry involving the vestibular and deep nuclei. Because of their position deep in the cerebellar white matter and the lack of complete understanding of the complex synaptology involving their neurons, a precise circuitry for these efferent cells has not been established. The Gymnotiform order of weakly electric fish do not have a deep cerebellar nucleus but rather a neuron within the cerebellar cortex, the eurydendroid cell, which has been shown to be efferent in nature (Finger, 1978b; Pouwels, 1978c; Carr et al., 1981). The caudal lobe of gymnotid species is believed to be intimately involved in coordinating this fish's body movements, utilizing the senses of electroreception (Heiligenberg, 1973; Maler, 1974), proprioception and vision (Bastian, 1975; 1976). Electrosensory information is relayed via the ELLL (Maler, 1979; Maler et al., 1981), and other brainstem nuclei to the caudal lobe. To understand the role of the cerebellum and the nature of their neuronal responses to external stimuli, this study sought to morphologically characterize all of the elements of this cerebellar lobe, and in particular, the eurydendroid cell. Through this study it is hoped that the caudal lobe can be viewed as a model system for interpreting the synaptology

of a cerebellar system and the role that the cerebellum plays in integrating sensory information. In particular, it is hoped that the morphology of the efferent eurydendroid neuron might enhance the understanding of the characteristics of the efferent system of the cerebellum.

MATERIALS AND METHODS

Three species of the Gymnotiform order were used in this study: *Eigenmannia virescens* ("transparent knife fish") of the family Sternopygidae; *Apteronotus albifrons* ("black ghosts") and another, known as "brown ghosts", both of the family Apteronotidae. The animals generally varied in length from 5 to 15 cms and were maintained in water with a resistivity of between 2-11 K Ω -cm and a temperature of 25-27°C. The following procedures were conducted during the course of the study:

I PERFUSION TECHNIQUE

To ensure maximum preservation for both light and electron microscopic investigations, all fish were perfused through the heart (Maler et al., 1981). Prior to fixation, the fish were deeply anesthetized with MS222 (Tricaine Methane Sulfonate). They were pinned to a block of Sylgard resin (Dow Corning no.184) exposing the lateral surface of the fish's body. The pericardial cavity was exposed so that a 30 gauge needle could be inserted into the conus arteriosus. To prevent backflow from the perfusion medium a small surgical clip (Yasargil clip, 4 mm) was placed behind the tip of the needle and clamped over a portion of the cardiac tissue to ensure that the needle remained within the conus. The atrium was then severed to permit drainage of the arterial system. The 30 gauge needle was attached via a thin, flexible plastic tubing to a two-way valve. The inlet ports of this valve were attached to two glass syringes - a 10 ml for saline and a 30 ml for fixative. Perfusion pressure was accommodated by a Sage syringe pump (Model 341) at nominal

flow rates of 0.1 to 0.4 mls/min. The flow rate was maintained so that the conus was swollen to no more than 3 times its original size. A small volume of saline solution (heparin (1 mg/15 ml); sodium nitrite (15 mg/15 ml); 0.96% NaCl; 0.042% KCl; 0.02% $MgCl_2 \cdot 6H_2O$; 0.028% $CaCl_2$ and 0.075% dextrose in a 0.05 M Tris buffer at pH 7.2), usually 2-5 mls, was initially passed through the circulatory system. The fixative, a 1% - 1% glutaraldehyde-paraformaldehyde solution (in 0.12 M sodium cacodylate at pH 7.2 with 2.5 mM $CaCl_2$) was then administered - 2 to 4 mls initially to give a rapid fixation then 15-30 mls at only 30% of the initial perfusion pressure. The total duration of the procedure from the time of opening of the pericardial cavity until the completion of the first 2-4 mls of fixative did not usually exceed 3 minutes, particularly for EM material where preservation is so critical. Following fixation, the cartilagenous skull cap of the fish was removed to expose the brain and the head stored in fixative for at least one hour before removing the brain from the cranial cavity. The complete procedure was performed under a Zeiss OpMi-1 operating microscope.

II STAINING FOR LIGHT MICROSCOPY

A. Following the perfusion of all brains used for light microscopy and Nissl staining, the fixative was removed and the tissue blocked and dehydrated through successive stages of ethanol immersion from 70%, 95% to 100%. Xylene rinses, to clear the alcohol, were done prior to the brain material being placed in hot paraffin. A vacuum oven was used to enhance paraffin penetration into the neural tissue.

Following orientation of the individual pieces in a plastic or paper boat the blocks were allowed to harden and then cut into a pyramidal shape. Sectioning of the tissue for light microscopy was performed on an American Optical microtome (model 820). Ribbons of paraffin pyramids containing serial sections of tissue were then mounted on glass slides, treated with xylene to remove the wax then rehydrated in 100%, 95% then 75% ethanol and finally distilled water. Cresyl violet staining and coverslipping completed the procedure for light microscopy.

B. Richardson's Stain. Sections of neural tissue embedded in plastic (see EM procedure) were made from .5 to 1 μ thick. The Richardson's procedure (Richardson, 1960) involved placing the plastic-embedded section on a slide smeared with Mayer's egg albumen and covered with a small pool of water. Heating the slide to 60-80°C stretched the sections flat and dried them completely. 1% periodic acid was dropped over the section for 5 minutes at room temperature. Following a rinse with distilled water, a pool of Mallory's Azur II-methylene blue (1% azur II in water; 1% methylene blue in 1% Borax solution) was placed over the tissue and heated for 5 minutes. After a rinse and brief xylene immersion the tissue was coverslipped.

C. The Golgi method used for staining was adapted from Valverde (1970). This is a triple impregnation procedure where pieces no larger than 3-4 mm thick are immersed in an aqueous solution containing 2.33% potassium dichromate and 0.19% osmium tetroxide. For each piece, a minimum of 20 mls of this solution was used. The tissue was stored at room temperature (18-25°C) for 7 days. The pieces were rinsed in a

small volume of 0.75% aqueous silver nitrate then stored in this same solution for 24 hours (minimum volume of 50 mls per piece).

The pieces were removed, blotted and again immersed in the osmium-dichromate solution, but for 6 days. After 48 hours in silver nitrate, 5 days in osmium-dichromate and 72 hours in silver nitrate, the tissue was dehydrated in absolute alcohol for 5 minutes and embedded in either paraffin or celloidin. Sectioning was done on an American Optical sliding microtome (Model 860) the pieces being cleared with clove oil prior to sectioning. Xylene was used to rinse the clove oil, and the sections were mounted on glass slides and coverslipped.

Golgi stained neurons and fibers were observed as well as drawn using a Zeiss microscope with a drawing tube apparatus. Measurements of somata, dendrites, fibers and dendritic spreads were made using a Leitz ocular filar micrometer calibrated with a stage micrometer. Photographs of Golgi-embedded, Richardson's stained and cresyl violet material was done on a Zeiss photomicroscope.

III ELECTRON MICROSCOPY

Tissue prepared for electron microscopy was perfused as previously described. Upon removal from the fixative, 100 μ sections were made of the cerebellar caudal lobe, using an Oxford vibratome (Model G), into a cooled solution of 0.12 M cacodylate buffer (pH 7.2).

Following 2-3 brief (1 min) washes in this buffer the tissue was immersed in a 2% osmium tetroxide solution, made in 0.12 M cacodylate buffer and 7% dextrose, for 2 hours at 4°C in the dark. Clearing the osmium

and cacodylate solution with water preceded "en bloc" staining of the tissue in 50% aqueous uranyl acetate for 1 hour at room temperature. Dehydration in 50% through 100% ethanol was then done prior to clearing of the neural tissue with propylene oxide (100%). To embed the material, a 12-14 hour immersion was performed in a 50%-50% mixture of propylene oxide and plastic [20 gm araldite resin 502; 13.6 gm DDSA (Dodecylsuccinic anhydride); 1.5% DMP-30 (Tri-dimethyl-amino-methyl-phenol)]. Following an immersion in fresh plastic for 1 hour, the tissue was oriented in Beem plastic capsules (J.B. EM, Montreal) and hardened through successive stages of heat - 2 hours at 40°C; 24 hours at 60°C and 6-8 hours at 90°C. Sectioning was done on an LKB Ultratome III (type 8801A) using glass knives made on the LKB Knife Maker (type 7801B). 100-200 μ square or hexagonal mesh grids (J.B. EM) were washed in 12 M acetic (glacial) acid, warm distilled water, then chloroform before being coated with a formvar film (0.5% in ethylenedichloride). All grids were carbon coated using an Edwards (model 4) carbon coating machine. Thin sections, grey silver in colour, were further stained using the Reynold's lead citrate (aqueous) method.

Material suitable for this study was examined and photographed using a Siemens (Elmiskop) 101 electron microscope. Photographic plates were developed in D-19 (Kodak) developer and fixed in Kodak rapid fixer. Negatives were enlarged using a Durst (D659) enlarger. The prints were made upon Ilfospeed 3.1 M and 4.1 M photographic paper.

RESULTS

I GROSS MORPHOLOGY

The cerebellum of the Gymnotiform order of weakly electric fish is comprised of three major subdivisions: i) the valvula cerebelli, ii) a large central cerebellar lobe called the corpus cerebelli, and iii) a vestibulo-lateral lobe, or archicerebellum (Maler et al., 1974). The vestibulo-lateral lobe can be further divided, based on its cell masses, into the eminentia granularis, the lobus caudalis principalis (LC) and the lobus caudalis pars medialis (LCpm). The caudal lobes bulge posterolaterally and appear to then curve ventro-medially as they rest on the dorsal surface of the brainstem. Ventral to the LC are the posterior lateral line lobes (PLLL) (Maler, 1979). Rostrally the caudal lobes are separated from the tectum by a deep oblique ventro-dorsal sulcus. Laterally, another sulcus demarcates the division between the ELLL and LC. It is separated superficially from the corpus cerebelli by a shallow horizontal fissure (fig. 1). Rostrally a distinct region (labelled x in figures 2a and 3) separates LC from corpus cerebelli. The granule cells of zone x are a direct dorsal continuation of the eminentia granularis and the molecular layer of zone x is continuous with the crista cerebellaris of n. medialis. Like the LC and eminentia granularis, zone x is positive for acetylcholinesterase and zone x is probably best understood as a part of eminentia granularis (fig. 3). The caudal lobes constitute slightly less than half of the cerebellum of weakly electric fish.

A. Laminar Organization

The laminar arrangement of the LC conforms with the classical description of other vertebrate cerebelli, except that it is inverted. In the transverse plane (fig. 2a, b) the most dorsal lamina is the granule cell layer which consists primarily of densely packed, small cells. Afferent fibers enter LC from its lateral aspect via the tractus PR. - Cer. (Maler et al., in prep.) and separate the LC granule cell mass into superficial and deep portions; the input to these two groups of granule cells is somewhat different (Maler et al., in prep.). On the basis of ultrastructural evidence which will be described in detail later, the LC granule cells can be further subdivided into lateral and central regions. Ventral and medial to the granule cell lamina is the molecular layer of the LC, which mediodorsally appears to be continuous with the molecular layer of the corpus cerebelli. The LC molecular stratum is entirely typical except for the presence of displaced Purkinje cells (see below). A narrow region is sandwiched between the granule cell and molecular lamina called the plexiform layer. The plexiform layer runs the full length of the LC and is about 100-200 μ in width. Straddling the midline of the caudal lobe molecular layer is another region of densely packed granule cells called the LC pars medialis (LCpm) (Maler et al., 1974). Some of these granule cells are displaced into part of the molecular layer; the displaced granule cells are located in the same volume of molecular layer which also contains displaced Purkinje cells. The LCpm contains only granule cells: neither Golgi nor Purkinje cells are found in this region. Zone x also has medially

located granule cells as well as dispersed granule cells, which extend through the molecular layer towards the eminentia granularis. The corpus cerebelli does not have any equivalent group of medial granule cells.

B. Extracerebellar Spaces

The LC is separated from the ELLL by a large space which is bounded caudally by the pigmented membrane which covers the entire gymnotid brain. This space is continuous, at rostral levels, with the IVth ventricle (fig. 4 - insert) and was previously identified as a ventricular space (Maler et al., 1974). It is clear, however, that the columnar endothelial cells of the ventricle do not continue into this infra-LC space; instead, it is bounded by the flat non-ciliated epithelial cells characteristic of pia (fig. 4; Peters et al., 1976). This space is therefore an expanded cerebellomedullary cistern and the connection with the IVth ventricle is analogous to the foramen of Magendie (Carpenter, 1978).

The important consequence of this reinterpretation is that the LCpm granule cells must be seen as resting upon a pial surface; the significance of this point will be discussed below.

II NEURONAL ELEMENTS AND AFFERENT FIBERS

A. Granule Cells

Granule cells of LC and LCpm have an entirely characteristic appearance of a small (4-6 μ m) round soma and three to six short thin dendrites which radiate out from the cell body to end in large globular or claw-like expansions (fig. 5). At the EM level granule

cells are seen to be grouped into clusters of six to twelve cells and have an ultrastructure similar to what has been described for other vertebrate orders (fig. 6). A single axon leaves the granule cell soma and bifurcates in the molecular layer to form a typical parallel fiber (Maler et al., 1974); the EM appearance of these fibers will be described below.

B. Mossy Fibers

Golgi impregnated mossy fibers can be observed coursing through both granule cell layers of the caudal lobe (fig. 7). Along their length they give off large complex terminals. The size of these terminals range from 2-5 μ in diameter. The mossy fibers appear to be predominantly longitudinally oriented since their lengths measured in the sagittal plane exceed by 3-4 times their lengths measured in the transverse plane.

Ultrastructurally, the mossy fiber is a heavily myelinated axon which dominates the axonal processes of the granular layers. Near their terminal boutons, the mossy fiber forms a large bulbous expansion that assumes a highly invaginated form (fig. 8). These terminals are observed terminating predominantly upon the dendritic claws of granule cells to form the classical glomerulus of all vertebrate cerebellae (Hillman, 1969; Larramendi, 1969). The dendritic claws typically contain a few cisternal elements and are moderately electron dense. Mossy fibers also occasionally terminate on thin electron lucent dendrites which are likely to belong to Golgi cells (see below).

Two distinct varieties of MF terminals appear to exist within the LC. One type has a very dispersed (d) complement of vesicles that sharply contrasts the more compact (c) vesicular distribution in the other type (fig. 9); otherwise these terminals are identical. Throughout both the caudal lobe and LCpm, these terminals appear to be roughly equal in number. Within each terminal are usually one or two central mitochondrial clusters, which is typical of such terminals in many other vertebrate species (Hillman, 1969; Palay and Chan-Palay, 1974). A few coated vesicles are observed in the vicinity of the presynaptic terminal where the small round vesicles tend to cluster. Occasionally, a coated pit appears as part of the pre-terminal membrane. Dense core vesicles are quite common in mossy fiber terminals and as many as 10-12 of these per terminal have been observed.

The mossy terminals are invaginated by the granule cell claws and asymmetric chemical synapses between them are usually located on the distal reaches of the finger-like extensions of the mossy fiber terminal. Gap junction contacts between mossy fibers and granule cell claws are infrequently seen. As in other vertebrates, Golgi cell axon terminals contact the outside of the granule cell dendritic claw.

Glomeruli of LCpm are similar to those of the caudal lobe except that there is much more interpenetration of the granule cell dendritic claw and the mossy terminal. In addition, Golgi cells are lacking here, and thus neither the dendrites nor axon terminals of this cell type take part in the formation of a glomerulus. The

granule cells dispersed in the molecular layer also make typical glomerular contacts and do not receive any input from the numerous nearby parallel fibers.

Through extensive HRP tracing experiments, Maler and Sas (personal comm.) have shown that the main sources of MF afferents to the caudal lobe are the lateral reticular nucleus and nucleus praeminentialis (nP). Lesions of the nP afferents to the LC resulted in extensive degeneration of the mossy fiber compacted (c) terminals and left the dispersed (d) terminals unaffected. These preliminary results indicate that the mossy fiber (d) terminals may originate from lateral reticular nucleus of the medulla.

C. Golgi Cells

The Golgi neuron is about 11 μ in diameter (table 1), and in cresyl violet material is found to be randomly distributed throughout the caudal lobe. Golgi material reveals two distinct populations of Golgi cells. There is a smaller cell that resides deep (d) in the granular layer (fig. 10), as well as a more superficial (s) type that is slightly larger and is positioned near the plexiform layer (fig. 11). The shapes of these subtypes are indistinguishable at the light and EM level. From a relatively round soma radiate 5-8 sinuously formed dendrites that ramify tortuously around the cell body. On a three-dimensional space these processes appear to form a sphere of average diameter 250 μ . Though the Golgi cell dendrite is uneven in width, the diameter of the primary processes is about .8 μ . Besides position and somatic diameter, the most obvious difference

between the two classes of Golgi cells is the ramification of their dendrites. The processes of the Golgi (d) are principally confined to the granule cell layer while those of the Golgi (s) extend into the plexiform lamina and shallow molecular layer as well as ramifying within the granule cell layer. Golgi axons are rarely visible but when observed, they appear to arise from either the cell body or primary dendrite, after which they richly branch near the Golgi neuron dendritic tree. Though Golgi cell terminals were found upon glomeruli (fig. 12a), some Golgi axons were found to course the full breadth of the granule cell layer to terminate in the plexiform lamina (fig. 12b). Serial sections of the caudal lobe reveal no pattern to the distribution of either the shallow or deep Golgi neurons.

In thin sections, Golgi cells have an appearance that is typical for vertebrate cerebella (fig. 13). The cytoplasm is electron dense and contains numerous ribosomes. Mitochondria, some rough endoplasmic reticulum (RER), Golgi apparatus and occasional dense lysosomes complete the complement of cytoplasmic organelles usually found in the Gymnotid Golgi cells. Synaptic contacts are occasionally seen on the soma of the Golgi cells.

The sinuous nature of the Golgi dendrite makes it difficult to identify at the EM level. Best observed in the plexiform layer where they course longitudinally before entering the molecular lamina (fig. 14), these processes have an ultrastructural appearance quite similar to classical descriptions of mammalian Golgi dendrites. Characteristically, they are much denser than other caudal lobe

dendrites with numerous longitudinally oriented microtubules, a few mitochondria and the occasional single neurofilament. Where the Golgi dendrite is observed in the plexiform and molecular layers, it is usually covered with parallel fiber contacts, typical of those upon Purkinje and eurydendroid processes in these two laminae (see below). Synaptic contacts by climbing fibers, which will be described in detail later, have not been observed on the few Golgi dendrites seen in the caudal lobe. Golgi axonal processes are heavily myelinated, and in the plexiform stratum they can sometimes be seen to make synapses "en passant" with eurydendroid dendrites or somata (see below). The most prominent position of the Golgi terminal, however, was that described for the glomerulus, where they are found on the external surfaces of the dendritic claw. No observable differences could be seen between the terminals of deep Golgi axons upon deep glomeruli and those boutons of shallow Golgi neurons upon either eurydendroid cells or superficial glomeruli.

D. Stellate Cell

Basket cells are not present in the caudal lobe and the stellate cell is only rarely seen. In Golgi stained material (fig. 15), this cell is found throughout the molecular layer. From a cigar-shaped soma emanate numerous thin sinuously formed dendrites that ramify in an elliptical fashion about the cell body. Ultrastructurally, the soma has a round eccentric nucleus with dispersed chromatin. There is a sparse array of organelles in the cytoplasm, typical of stellate neurons of higher vertebrates, but in contrast to the mammalian

stellate cell, the cytoplasm of the few cells viewed was electron dense. It was not possible to identify stellate cell dendrites with any certainty; an unusual axonal process was occasionally observed synapsing upon dendrites within the molecular layer. This pleomorphic terminal was electron dense consisting almost entirely of flattened vesicles and few mitochondria and we believe it to belong to the stellate cell.

E. Purkinje Cell

The Purkinje (P) cell is the largest of the cerebellar neurons (Table 1). In cresyl violet sections these cells are predominantly located in or near the plexiform lamina but can also be displaced deep within the molecular layer. The cell body is round to slightly ovoid and is usually oriented with its long axis perpendicular to the plexiform layer. Golgi impregnated material shows that the dendritic arborization of the P-cell usually originates from a single stalk at the apical pole (fig. 16). The primary dendrites fan out in an orientation that is mostly planar in the sagittal field often extending the full rostrocaudal extent of the molecular layer (950 μ). However, some spreading of these processes is in the transverse plane as well (200 μ). Some smaller, secondary dendrites and even a few tertiary ones arise from the primary branches. It was often observed that the dendrites curve backwards towards the plexiform layer which is uncharacteristic of higher vertebrates but common to many teleost species (Larsell, 1967). Both large and small dendrites are extremely spinuous. The Purkinje dendritic tree often extends the full height of the molecular lamina of the caudal lobe, but does not

extend into the subjacent posterior lateral line lobe, (ELLL). At both light and EM levels no observable physical barrier was seen at the junction of the ELLL and the caudal lobe, where dendrites from neurons of both brain regions abut while axonal elements (parallel fibers) continue to penetrate in an effortless fashion. Purkinje axons and their collaterals arise from the basilar pole of the soma and ramify primarily in the plexiform layer. In Golgi material, these collaterals can be seen to possess numerous terminal enlargements along their length but the extent of the collateral axonal plexus is difficult to estimate because complete impregnation of these myelinated axons has not been achieved.

Ultrastructurally, the Purkinje soma assumes a dominant presence among the neuronal elements of the caudal lobe. The apical dendrite is often observed leaving the large soma in the direction of the molecular layer oriented in the perpendicular fashion that was suggested at the light microscope level. The P-cell has a slightly eccentric nucleus consisting of dispersed chromatin and a large dense nucleolus, usually located in close apposition to the nuclear membrane. The cytoplasm is electron dense containing a population of organelles that is typical of all vertebrate Purkinje neurons (fig. 17). The perikaryon is slightly rugose in appearance and is almost completely free of synaptic input; on very rare occasions one may find a Purkinje cell axon collateral synapsing upon a Purkinje cell soma. Surrounding the cell body is a thin glial sheath that is a characteristic feature of P-cells. The cytoplasm contains

numerous free ribosomes which impart much of the cell density. As well, there is a full complement of RER, Golgi apparatus, mitochondria and some dense lysosomes. The large nucleus appears to be positioned in a pivotal point around which the organelles are suspended in a "whorl-like" fashion, described similarly for the mammalian P-cell (Palay and Chan-Palay, 1974). A characteristic feature of Purkinje neurons in general, and of those in the caudal lobe as well, is the presence of numerous sublamellar cisternae which are apposed to the cell membrane. The dendritic processes of the Purkinje cell of the caudal lobe are clearly distinguishable from all other molecular layer dendrites because of their high spine density, typical of Purkinje dendrites, throughout the vertebrate phyla. In transverse and sagittal planes, both the large and small dendrites display this preponderance of spines which receive typical parallel fiber input (to be described below). Some cisternal material exists within these Purkinje spines as well as within the main dendritic processes, and in addition have numerous mitochondria and a longitudinal arrangement of microtubules. In cross-section these microtubules appear to establish an organized lattice network; neurofilaments are only rarely present in the dendrites of the P-cell. Purkinje cell axon collaterals emit terminal boutons, which contain a pleomorphic vesicle population, consisting of both flat and round small vesicles (fig. 18). Within the Purkinje terminal an occasional dense core vesicle as well as a few coated vesicles may be seen. The synaptic articulations of the P-cell axon terminals will be described later.

Input to Purkinje Cells

a) Parallel fibers

Purkinje cell dendritic spines receive classic parallel fiber synaptic input. The small parallel fiber terminals contain a homogeneous population of a few rounded vesicles. They make asymmetric contacts on the lateral surfaces of most Purkinje spines of primary, secondary and tertiary dendritic processes (fig. 19a).

b) Climbing fibers

Optimal Golgi impregnations have revealed myelinated axons running in the plexiform layer which turn ventrally to ramify in the molecular layer. These fibers typically wind round and climb upon the dendrites of both Purkinje cells and eurydendroid cells (see below), and can thus be firmly identified as climbing fibers; retrograde tracing experiments have shown that these climbing fibers derive from a typical inferior olivary nucleus (Maler and Sas, personal comm.). Climbing fiber terminals are variable in size and many can be as much as 3-4 times the diameter of the parallel fiber boutons (e.g. 3 μ in diameter). At the electron microscope level the large size of the climbing fiber was the main criterion for identifying it as compared to the smaller parallel fiber terminals (fig. 19b). We have not found any climbing fiber collaterals to the granule cell layer. It is obvious that one climbing fiber may contact adjacent Purkinje and eurydendroid dendrites. Neither Purkinje cells nor eurydendroid cells are perfectly planar, and we therefore cannot say with assurance that one Purkinje and eurydendroid cell receives input from only one climbing fiber. Ultrastructurally, climbing fiber boutons were first identified on the basis of their size.

Climbing fibers have some very characteristic features that further distinguish them from parallel fibers at the EM level (Palay and Chan-Palay, 1974). The most prominent of these is their vesicle population. In addition to very numerous small round vesicles, a single climbing fiber bouton may contain as many as 6-10 dense core vesicles as well as numerous large empty vesicles with the same diameter as the dense core vesicles (fig. 19c, d). The dense core vesicles may be on the order of 2-3 times the diameter of the typical small vesicle. Occasionally a dense core vesicle may be seen close to a cluster of small vesicles at the pre-synaptic membrane.

Unlike the parallel fiber bouton, the climbing fiber is fairly electron dense, and in addition, contains a number of mitochondria and cisternal elements. Whereas the parallel fiber usually only contacts one Purkinje spine, one climbing fiber terminal has been observed to contact several spines. The climbing fiber synapse is asymmetric with a well developed postsynaptic density. In Golgi-impregnated material, the distinction in size between parallel fibers and climbing fibers is clearly evident (fig. 20).

F. Eurydendroid Cell

The eurydendroid cell represents the output element of the teleost cerebellum (Finger, 1978b). Unlike the situation in higher vertebrates, these cells are not clustered into nuclear groups but are found within the cerebellar cortex itself (Finger, 1978b; Pouwels, 1978c). In the gymnotid caudal lobe there are two major classes of eurydendroid cells: those found in the plexiform layer (eurydendroid (pl) which are by far the most numerous, and some that are found

within the granule cell layer (eurydendroid (gr)); it must be emphasized that the eurydendroid cells are not found in the LCPm.

Eurydendroid cells of the plexiform layer:

These cells can be readily distinguished from nearby Purkinje cells at both light (fig. 21) and electron microscopic (fig. 22)

levels:

a) eurydendroid cells are much paler than P-cells in both Richardson-stained sections and at the EM level.

b) eurydendroid cells have strongly bipolar somata with their long axis parallel to the plexiform layer and thus orthogonal to the axis of the P-cell (see above).

Eurydendroid cells are often grouped into clusters of 2-4 cells and these clusters are in turn associated with a small number of P-cells. The eurydendroid (gr) neurons were far less common numerically but their morphology was virtually indistinguishable from the eurydendroid (pl) cells.

Although we did not feel that it warranted further subdivision, there is a difference between medially and laterally located eurydendroid cells. The lateral ones have very roughened somata with numerous irregular protrusions and complex spines (fig. 23), while the more medial eurydendroid somata are fairly smooth and have only a few spines (fig. 24). This characteristic feature was consistent with eurydendroid neurons in both the plexiform and granule cell layers.

Two or three thick dendrites arise from the poles of the eurydendroid (pl) cell; as they run within the plexiform lamina

these dendritic trunks give off a few branches which ramify sparsely within the granule cell layer adjacent to the plexiform layer. The dendritic trunks soon bend toward the molecular layer (i.e. ventrally) and commence to branch profusely into secondary and tertiary branches. The latter branches are quite long and run in a sinuous fashion. The tertiary branches are exceedingly thin ($.5 \mu$) but do not appear to taper. The primary dendrites are usually smooth and spines are only rarely seen on the secondary and tertiary dendrites. The overall spread of the eurydendroid (pl) cell dendritic tree in the molecular layer is similar to that of the P-cell in that it is fairly planar with the long axis oriented sagittally. Our impression from favourably sectioned Golgi material is that the eurydendroid cells' dendritic arbor may have an even greater rostro-caudal extent than that of the P-cell (fig 24, insert).

Eurydendroid cells have a large eccentric nucleus with a peripherally located nucleolus and dispersed chromatin (fig. 25). Ribosomes and RER are not as abundant as in the Purkinje cell, and unlike the P-cell, the various cytoplasmic organelles are not organized into swirls around the nucleus. Sublamellar cisternae are rarely observed in the eurydendroid cell. The complex somatal protuberances and spines found on the lateral eurydendroid cells are very distinctive as they contain an extensive network of cisternal elements, occasional sacs with dense cores, and often receive the Purkinje cell axon terminals that can be found contacting smooth portions of the cell body (fig. 26).

A very prominent feature of the eurydendroid dendrite, clearly distinguishing it from Purkinje cell processes, are the numerous fascicles of neurofilaments running axially within the dendrite along with microtubules. The axon hillock and initial segment of the eurydendroid (pl) cell (see fig. 18) are entirely typical of most neurons. We have not been able to follow the thickly myelinated axons of this cell for any distance in either Golgi preparations or EM and cannot ascertain whether it gives off any local collaterals.

III SYNAPTIC INPUT TO THE EURYDENDROID CELL

The smooth secondary and tertiary dendrites of the eurydendroid (pl) cell receive innumerable contacts from parallel fibers (fig. 27a). As indicated earlier, climbing fibers are often observed contacting eurydendroid dendrites (fig 27b). This was visible in Golgi-impregnated sections where the climbing fibers entwined the eurydendroid dendrites in much the same fashion as with the Purkinje cell dendrites. Quite noticeable at the EM level were the frequent contacts of climbing fiber terminals upon thinner secondary and tertiary branches.

Within the granule cell layer we have occasionally observed what we believe to be eurydendroid dendrites receiving mossy fiber input. These dendrites assume a similar morphology to the granule cell claw but appear electron lucent and contain neurofilament fascicles. They appear to participate in some glomeruli with granule cell dendrites. It is not possible to distinguish at the EM level, between dendrites of eurydendroid cells in the granule cell and plexiform laminae, but

from observations of Golgi material we conclude that when such processes are near the plexiform layer they more often stem from eurydendroid (pl) cells.

The soma and thick proximal dendrites of the eurydendroid (pl) are liberally contacted with one morphological class of terminal. Since the Golgi material showed that Purkinje cell axons ramify extensively in the vicinity of the eurydendroid (pl) neurons, we conclude that the dominant input to the eurydendroid (pl) somata is from Purkinje cells (see Fig. 25 and 26). The Purkinje cell terminal generally contains a central core of mitochondria and a dispersed complement of pleomorphic vesicles in a medium-sized bouton. The synaptic contacts upon eurydendroid (pl) cells are clearly symmetrical, and the terminals conform closely to those described for Purkinje cells in other vertebrates (Llinas, 1969; Palay and Chan-Palay, 1974). The density of Purkinje cell input to the eurydendroid (pl) cells is high. In a single thin section 6 to 12 terminals may be seen contacting one soma. The somatic spines and protrusions, particularly those of the lateral eurydendroid (pl) cells, are especially densely innervated. Occasionally pleomorphic terminals that are morphologically dissimilar to the more typical Purkinje cell bouton, are observed contacting eurydendroid (pl) cell bodies and dendritic processes in the plexiform layer. Containing either a more dense or a less dense vesicular population than Purkinje terminals, these are believed to be stellate cell and Golgi cell boutons, respectively.

The eurydendroid cells of the granule cell layer have generally smaller and rounder somata than their counterparts in the plexiform lamina (Table 1). Two to three stout primary dendrites, giving off thin secondary and tertiary branches, ramify sparsely in the granule cell layer. Often the primary dendrites will enlarge forming "frond-like" excrescences similar to those described in the mammalian cerebellum (Chan-Palay, 1977). The dendrites are not planar and often extend to the molecular layer of widely separated cerebellar areas such as the caudal lobe and corpus cerebelli. Most Golgi impregnations reveal only a small portion of the dendritic tree of this cell type and so neither the full extent of the arbor nor the possibility of eurydendroid (gr) subtypes could be determined. In one fortunate instance we observed a eurydendroid (gr) cell with its soma in the caudal corpus cerebelli; and one dendritic tree ramified in the caudal lobe granule cell layer, adjacent corpus cerebelli, and the molecular layer of zone x as well. A second dendrite travelled about 100 μ rostrally to ramify in the molecular layer.

At the ultrastructural level the eurydendroid (gr) somata appears identical to that of the eurydendroid (pl). It is not possible to differentiate between the dendrites of these two types of eurydendroid cells in the EM.

Somata of the eurydendroid (gr) are usually free of synaptic contact and although there is no direct evidence it is assumed that the eurydendroid (gr) dendrites in the molecular layer receive an

input (Purkinje cell terminals, parallel fibers, climbing fibers) similar to that of the eurydendroid (pl) dendrites.

IV AN UNUSUAL CEREBELLAR INPUT - THE TRACT OF THE STRATUM FIBROSUM

A fiber system - the tract of the stratum fibrosum (T.St. fib.) enters the ELLL from its rostro medial aspect and terminates in the ventral molecular layer of that structure (Maler, 1979; Maler et al., 1981); T.St. fib. preterminal fibers and their terminals closely resemble classic parallel fibers (see ref. above). At the lateral aspect of the ELLL (lateral segment, Maler et al., submitted; Heiligenberg and Dye, in press) collaterals of this tract turn dorsally and invade the overlying LC (fig. 28). Golgi impregnations suggest that these fibers terminate as parallel fibers in the lateral part of the caudal lobe in that part of the molecular layer adjacent to the granule cell layer; note that if the caudal lobe were in a normal cerebellar orientation this would correspond to ventral molecular layer, i.e. the same as this T.St. fib. terminal zone in the ELLL. Recent anterograde and retrograde studies have demonstrated that T.St. fib. originates in the nucleus praeeminentialis and have confirmed that it sends collaterals to the ventral molecular layer of the lateral caudal lobe (Maler and Sas, personal comm.). Insofar as we know, this is the only example of a parallel fiber-like input to the cerebellar molecular layer which does not arise from granule cells but rather from an extracerebellar source.

In the ELLL, T.St. fib. terminals can be distinguished from classic parallel fiber terminals at the EM level only by their slightly increased electron density and more highly packed vesicle population (Maler et al., 1981). We used the same criteria to identify T.St. fib. terminals in the caudal lobe. Asymmetric synaptic contacts, similar to those made by parallel fibers were seen between T.St. fib. boutons and both Purkinje cell dendritic spines and eurydendroid cell smooth secondary dendritic branches. The T.St. fib. terminals were, however, only a small fraction of the classic parallel fibers boutons present in the same area.

DISCUSSION

I COMPARATIVE AND DEVELOPMENTAL ASPECTS

The results presented in this study at both the light and electron microscopic level demonstrate that the caudal lobe of these species of the Gymnotidae order is organized, for the most part, as a classic cerebellar cortex. Exceptions to the cerebellar plan will be touched upon below. The cerebellar caudal lobe is a simple laminar structure. Although inverted with respect to the cortical strata of the mammalian cerebellum (Larsell, 1952), the laminae are found quite characteristically as the granule cell layer, the molecular layer and an intermediate, or plexiform layer. The caudal lobe of these fish emanates as a posterolateral bulge from the main portion of the cerebellum in much the same fashion as does the archicerebellum of higher vertebrates (Carpenter, 1978). Within the weakly electric fish, the relationship between the caudal lobe and other components of the archicerebellum, namely the eminentia granularis and zone x, is still obscure. Recent studies on mormyrid fish, another teleost, have emphasized the continuity of the eminentia granularis and the caudal lobe by referring to the latter as the eminentia granularis pars posterior (Libouban and Szabo, 1977). Implicit in this nomenclature is the notion of an evolutionary link between the eminentia granularis and the caudal lobe. Though precise embryological work is lacking in this respect, the hypothesis that the caudal lobe (electroreceptive) of the Gymnotiform order has evolved from the eminentia granularis (lateral line) in parallel with the evolution of the posterior lateral line lobe (electroreceptive)

from the nucleus-medialis (lateral line), is favoured. Since there is insufficient evidence for this point of view, and on purely historical grounds, the term "caudal lobe" is retained. Zone x is included in the archicerebellum because of its positive reaction to cholinesterase stain and the continuity of its molecular layer with the crista cerebellaris (Maler, unpublished observations). At this point it is not possible to ascertain whether zone x merely represents a caudal continuance of the eminentia granularis or whether it is a separate lobule intermediate to the caudal lobe and the corpus cerebelli. In view of the visual-electroreceptive interactions discovered principally in the rostral caudal lobe (Bastien, 1975), these distinctions may prove to have functional significance. Careful examination of his figure 2 indicates that the positions of the bimodal response units recorded by the electrodes were more likely to be in the caudal corpus cerebelli or zone x. It is clear that further resolution to this problem requires precise histological identification of the recording sites in the archicerebellum.

The presence of a medial granule cell mass, LC pm, has been a puzzling feature of the gymnotid cerebellum (Maler, 1974) as well as that of other teleosts (Pouwels, 1978; Finger, 1978a). During normal cerebellar development in higher vertebrates, a mass of neuroblast cells forms an external germinal layer that resides just below the pia surface (Miale and Sidman, 1961). This external

germinal layer is usually a temporary structure since its cells serve as precursors for the granule cells (Altman and Bayer, 1978). Even in normal development it may happen that granule cells of this layer differentiate in situ or en route to the granule cell layer. Under these circumstances they form clusters of displaced or "ectopic" granule neurons within the molecular layer or just beneath the pial surface (Chan-Palay, 1972; Palay and Chan-Palay, 1974). These ectopic granule cells have been shown to maintain the synaptic morphology typical of glomeruli in the proper granule cell layers (Chan-Palay, 1972; Stoughton et al., 1978). Since the pars medialis caudal lobe (LCpm) has been identified as being on a pial surface (figure 4) we propose that it is a group of unmigrated or "ectopic" granule cells. In this respect, granule cells found in the molecular layer between the LCpm and the proper LC granule cell layer would be considered arrested during the course of their migration. Consistent with this point is the absence of large neurons in the LCpm. Purkinje, Golgi and deep nuclear neurons do not stem from the external germinal layer and are not found within it during development (Altman and Bayer, 1978; Gould and Rakic, 1981). These arise from the neuroepithelium. It is interesting that, in the gymnotid caudal lobe, displaced Purkinje neurons have been observed in the molecular layers containing the ectopic granule cells. If this correlation is not fortuitous it might indicate that some common factor is perturbing the migration of both cell types. The LCpm and displaced Purkinje neurons are featured only in the archicerebellum. The LCpm must have some

functional significance since its granule cells appear to receive normal mossy fiber input and then project their parallel fibers to very specific recipient zones (Maler et al., in preparation). Certainly it is interesting that a component of the archicerebellum of mammals, the flocculus, has been shown to be slower at developing than the other parts of the cerebellar cortex (Altman and Bayer, 1978). Comparative and developmental work in the archicerebellum of teleosts may offer some clues as to the cellular mechanisms which control neuronal, and in particular, granule cell migration.

II MORPHOLOGICAL CORRELATES

Afferent Pathways. The mossy and climbing fiber input to the caudal lobe and their morphological relationships with the neuronal elements of the gymnotid cerebellar cortex conforms remarkably to the vertebrate scheme (Larsell, 1967; Palay and Chan-Palay, 1974). The projection of the tract of the stratum fibrosum fibers (figure 28), directly to the molecular lamina of the caudal lobe (Maler and Sas, unpublished observations) is a considerable departure from the more orthodox afferent pathways. A quantitatively minor source, this input must be confirmed in other teleosts and for the purposes of this discussion its significance will be disregarded.

Following a similar criterion of vesicle compaction, two classes of mossy fiber terminals reported within the mammalian cerebellar cortex (Palay and Chan-Palay, 1974), have also been identified in the granule cell layer of the gymnotid caudal lobe. Preliminary investigations suggest that the more compacted variety of mossy fiber terminal arises from the *n. praeeminentialis*.

An interesting parallel exists between the ultrastructure of the glomeruli in lower vertebrates as compared with those identified in mammals (Chan-Palay, 1972; Sotelo and Llinas, 1972) - that being the presence of two types of synaptic formations, conventional synaptic zones and gap junctions.

Climbing fibers of the gymnotid cerebellum bear striking resemblance to those identified in other vertebrates (Sotelo, 1969; Larramendi, 1969). Their larger size clearly distinguished them from the other prominent terminals of the molecular layer, namely parallel fibers. Attention must be drawn to the numerous large dense core vesicles and large empty vesicles found within the climbing fiber terminal. This contrasts parallel fiber terminals which contain only small clear vesicles. Dense core vesicles have been previously reported in climbing fibers (Larramendi, 1969; Sotelo, 1969; Palay and Chan-Palay, 1974) but have not generated much interest.

Recent studies have indicated that some neurons may contain more than one transmitter (Pelletier et al., 1981). In light of the complex role generally assigned to climbing fibers (Baker and Berthoz, 1977; Bender, 1980) it may be important to determine whether the clear and dense core vesicles of climbing fibers contain different neuroactive substances.

III NEURONAL ELEMENTS CHARACTERISTIC OF THE CEREBELLAR CORTEX

Comparative analyses of the morphology of most cells of the gymnotid caudal lobe suggests that there is a remarkably conservative element within the neurons of the vertebrate cerebella. Granule cells, Golgi cells, Purkinje neurons and stellate cells of this teleost cerebellum are all prominent within the cortex of the mammalian homolog (Larsell, 1967; Palay and Chan-Palay, 1974). Notably absent from the Gymnotiform order as well as other teleosts, is the basket cell (Nieuwenhuys and Nicholson, 1969a, b; Pouwels, 1978; Finger, 1978a).

Granule cells are the smallest and most numerous, characteristic of the mammalian cerebellum. Comparative cerebellar morphologies of the vertebrate phyla reveal little deviation from the classical granule cell descriptions of Cajal (1955). Evidence for some nonbifurcating parallel fibers has been reported in some teleosts (Pouwels, 1978b). However, no evidence of such a nature was observed in the molecular layer of the gymnotid caudal lobe. The synaptic relations of granule cells and mossy fibers appear well preserved, even for the "ectopic" neurons, as previously discussed. A feature that was not very conspicuous at the light microscope level, yet quite noticeable ultrastructurally, was the clumping of granule cells into groups of 4 to 6 perikarya, a feature also noticed in the mammalian cerebellar cortex (Palay and Chan-Palay, 1974). Any relevance or connection between this observation and the parasagittal zonations observed for both afferent and efferent fibers in the cerebellum of higher vertebrates (Groenewegen et al., 1979; Voogd and Bigarre, 1980) remains to be tested.

Stellate neurons of the gymnotid caudal lobe and those described for other vertebrates feature few uncommon characteristics (Larsell, 1967; Uchizono, 1969) except for the fact that in the weakly electric fish of this study, few stellate neurons were present in the caudal lobe molecular layer. A neuron that was a most prominent element of the cerebellar cortex in these fish and one that bears remarkable conformity to similar descriptions in higher vertebrates is the Golgi cell. Identification of the Golgi axonal terminals upon the periphery of glomeruli within the granule cell layer of the caudal lobe intimates that their involvement as an inhibitory interneuron is consistent with previous observations in other vertebrates (Hamori and Szentagothai, 1966; Eccles et al., 1966). Categorically, a bimodal distribution of somatal diameters of Golgi cells in the weakly electric fish, alludes to the consistency in classification of the small, deep varieties and large superficial types described for mammals (Palay and Chan-Palay, 1974). Considering the differences in positions of dendritic arborizations, it is conceivable that the deeper Golgi cell, with its processes mostly confined within the granule cell layer, has a different functional role to play than the more superficial type that extend their dendrites mostly into the molecular layer.

Purkinje cells are perhaps the most characteristic cerebellar feature of most vertebrates. They are equally prominent in the caudal lobe of the gymnotid fish. Their characteristic spiny dendritic appendages have been described in many other cerebella (Cajal, 1955; Larsell, 1967; Uchizono, 1969). Typical of the Purkinje neurons of

other primitive vertebrates (Larsell, 1967; Pouwels, 1978b) those of the caudal lobe of these fish rarely display branching beyond the tertiary level. Two rigorous biochemical criteria are available for identifying Purkinje cells. One is that these neurons contain glutamic acid decarboxylase (GAD), the enzyme responsible for synthesizing the Purkinje cell neurotransmitter, γ -aminobutyric acid (GABA) (Oertel et al., 1981; Kuriyama et al., 1966); the second is that Purkinje neurons contain vitamin-D dependent calcium binding protein (CaBP) (Baimbridge and Miller, 1982). Cells within the caudal lobe of the Gymnotiforms, identified as Purkinje neurons, have been shown to be immunoreactive to the antibody raised for CaBP (Jande et al., 1982). Neurons believed to be Purkinje cells of the caudal lobe were found to fluoresce indicative of the immune reactivity to the GAD antibody used to identify GABAergic neurons in the ELLL (Maler et al., in preparation). Thus it may be concluded that the caudal lobe of these weakly electric fish, contains a primitive version of the Purkinje cell identified in so many other vertebrates (Larsell, 1967; Palay and Chan-Palay, 1974).

IV EURYDENDROID CELLS AND FUNCTIONAL ASPECTS OF THE CAUDAL LOBE CIRCUITRY

At first glance the eurydendroid cell, the teleost cerebellar output neuron (Nieuwenhuys et al., 1974; Finger, 1978; Carr et al., 1981) appears to be an exception to the higher vertebrate plan. If figure 29 is consulted, however, it becomes clear that the eurydendroid

cell receives only one anomalous input. In the higher vertebrate scheme, a climbing fiber will contact several Purkinje neurons and send collaterals to cells of the deep cerebellar nuclei. The Purkinje cells will then project to those deep nuclear cells innervated by the collaterals of their own climbing fiber output (Groenewegen et al., 1979; Dietrichs, 1981). In the gymnotid caudal lobe a climbing fiber will contact a Purkinje cell(s) and the overlapping apical dendrites of an adjacent eurydendroid neuron(s). The Purkinje cell(s) will contact neighbouring eurydendroid cell(s) with which they presumably share climbing fiber input.

In higher vertebrates, mossy fibers contact granule cells and send collaterals to deep nuclear neurons (Ekerot and Larson, 1973; Chan-Palay et al., 1977). Granule cells then contact Purkinje cells which in turn, project to those deep nuclear neurons innervated by the same mossy fibers (Dietrichs, 1981). In the gymnotid caudal lobe, mossy fibers establish the same circuit since they contact eurydendroid cells directly on that part of their dendritic tree that ramifies in the granule cell layer, perhaps in intimate association with dendritic excrescences. Mossy fibers project indirectly to eurydendroid cells via the granule cell-parallel fiber system (dotted line in figure 29) which is the major difference between the gymnotid caudal lobe and higher vertebrate cerebellar circuitry. It is important to note that neighbouring Purkinje and eurydendroid cells will share a similar input from parallel fibers and that Purkinje neurons will synapse mainly upon neighbouring plexiform layer eurydendroid cells with which

they share a common input. Thus this superficially unusual pathway appears to be functionally similar to the circuitry of the higher vertebrate cerebellum. The use of granule cells as interneurons between afferent mossy fibers and efferent eurydendroid cells parallels the interneuronal relationships present within the dentate nucleus of mammals (Chan-Palay, 1977). In the gymnotid caudal lobe, eurydendroid cells are also contacted directly by Golgi and stellate neurons. Although these connections are quantitatively very minor, it is evident that the distinction between interneurons of the cerebellar cortex and of the output neurons disappears since Golgi, stellate and even granule cells serve both roles.

Although the deep nuclear cells of the mammalian cerebellum are the predominant output source, their complex anatomy has meant that their physiological study lags far behind that of the cerebellar cortex. Since Purkinje cells and eurydendroid (pl) neurons share the same simple parallel fiber input, and since this input is directly from the periphery (Bastian, 1975; Maler et al., in preparation), it may be easier to analyze what the cerebellum as a whole is doing by using the caudal lobe as a model system. The results of Bastian (1975) lend themselves to a straightforward interpretation in terms of the circuitry described above. Bastian stimulated the fish with small plates moving parallel to the fish's body along its longitudinal axis. He described, on physiological grounds, two major cell types of the caudal lobe:

a) some neurons responded in an excitatory fashion when the electrosensory mechanisms of the fish were stimulated by passing a

metal plate over a wide area of skin - the shape of this excitatory receptor field being a dorso-ventral strip.

b) other cells responded in an excitatory fashion similar to a) but there were inhibitory flanks on the sides of the central excitatory zone.

One interpretation of Bastian's results is that these units represent the two largest neuronal elements of the caudal lobe Purkinje and eurydendroid (pl) cells, respectively. Based on the knowledge of the underlying morphology of the caudal lobe, as described in this study, we propose that the first type of unit is a Purkinje cell with its excitatory response due to parallel fiber input to its dendritic tree. The second type of unit would correspond to the eurydendroid (pl) cell with its excitatory response due to the same parallel fiber input and its inhibitory flanks due to adjacent Purkinje cell inhibition of the eurydendroid (pl) neuron. The size and orientation of the receptive fields is also consistent with the topography of the caudal lobe input (Maler et al., in preparation). This hypothesis has two testable consequences:

i) the eurydendroid (pl) cell projects to the torus semicircularis whereas Purkinje neurons do not (Carr et al., 1981). Therefore the unit (b) with inhibitory flanks should be the only one antidromically activated by stimulation of the torus semicircularis; and ii) since Purkinje neurons employ GABA (Kuriyama et al., 1966) as a transmitter, it should be possible to eliminate the inhibitory flanks of the eurydendroid (pl) cell by local iontophoresis of GABA blockers, such as bicuculline.

This line of reasoning may not apply to the eurydendroid cells within the granule cell layer. Their very divergent branching pattern implies that these neurons may integrate more disparate inputs than the eurydendroid (pl) types. It is interesting that both classes of eurydendroid neurons have different projection sites (Carr et al., 1981). There is a functional parallel here to the deep nuclear cells of the mammalian cerebellum. These also contain certain neurons which receive input from widespread cortical regions as well as others with more restricted inputs; and these cells probably have different projections (Chan-Palay, 1977). An important consideration is that the caudal lobe fosters more than one morphological type of eurydendroid neuron indicated by the rugose nature of the lateral varieties. Certainly more than one cell type exists in the deep nuclei of the mammalian cerebellum (Chan-Palay, 1977). To divide the caudal lobe into functional zones, however, would require a precise physiological examination with respect to its medial and lateral areas.

Figure 30 summarizes the circuitry and neuronal elements inherent within the caudal lobe of the gymnotid cerebellum. When viewed in conjunction with the line diagrams of figure 29, it is evident that with one or two exceptions, the synaptology of the archicerebellum of the weakly electric fish, Gymnotidae, closely resembles the plan documented by others of the vertebrate phyla.

Abbreviations:

ax	- axon
CC(Cb)	- corpus cerebelli
cf	- climbing fiber
d	- dendrite
DCN	- deep cerebellar nuclei
dcv	- dense core vesicle
des	- desmosome
EG(EmGr)	- eminentia granularis
EM	- electron microscope
eur	- eurydendroid (general)
eur(gr)	- eurydendroid cell of the granule cell layer
eur(pl)	- eurydendroid cell of the plexiform layer
f	- foramen
GA	- golgi apparatus
GCL	- granule cell layer
GJ	- gap junction
gl	- glomerulus
Gol	- Golgi cell (general)
Gol(d)	- deep Golgi cell
Gol(s)	- superficial Golgi cell
gr	- granule cell
h	- hillock
LC	- caudal lobe
LCpm	- 'pars medialis' caudal lobe
mf	- mossy fiber (general)

Abbreviations (Cont'd)

mf(c)	- mossy fiber (compact)	}
mf(d)	- mossy fiber (dispersed)	
mit	- mitochondria	
MOL	- molecular lamina	
mt	- microtubule	
nc	- nucleolus	
nf	- neurofilament	
NLLa	- anterior branch of the ant. lateral line nerve	
NLLp	- posterior branch of the ant. lateral line nerve	
nPr.	- nucleus Praeeminentialis	
nu	- nucleus	
nXs	- sensory nucleus of vagus	
pf	- parallel fibers	
PFL	- plexiform lamina	
ELLL	- posterior (electrosensory) lateral line lobe	
Pur	- Purkinje cell	
rer	- rough endoplasmic reticulum	
s	- sulcus	
sp	- spinal cord	
st	- stellate cell	
Te0	- optic tectum	
Tr.Pr.-Cer.	- tractus praeeminentialis-cerebellaris	
T.St.fib.	- tract of the stratum fibrosum	
v	- ventricle	
X	- zone x	
μ	- micrometer	

TABLE 1

Mean somatal diameters of cells in the caudal lobe:*

CELL TYPE	DIAMETER: (μ)
Granule Cell	5.4
Golgi cell (deep)	10.8
Golgi cell (superficial)	11.9
Stellate cell	12.5 x 8.8
Purkinje cell	20.6
Eurydendroid (pl) cell	17.0 x 9.8
Eurydendroid (gr) cell	14.9 x 9.8

*Except for the stellate neuron, measurements were based on a minimum of 15-20 cells; only 3 stellate cells were observed

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FIGURE 1

Gross morphology of the gymnotid brain showing the posterolateral bulge referred to as the caudal lobe. Dorsal to the caudal lobe lies the corpus cerebelli while ventrally rests the PLLL, considered part of the brainstem area (Maler, 1979).

FIGURE 2

Cresyl violet stained transverse sections through the a) rostral portion of the caudal lobe and, b) caudal part of the caudal lobe.

a) note zone χ as part of the caudal lobe, rostrally, and the division between corpus cerebelli and the caudal lobe by the sulcus marked by an arrow. Although the MOL of zone χ and the caudal lobe appears uniform, both show a division between the MOL of the ELLL and the corpus cerebelli. Note the presence of the rostral LCpm and neurons scattered through the MOL of the LC.

b) though zone χ no longer is apparent, the caudal lobe displays its prominent lateral bulge and is distinctly separate from the ELLL, ventrally and the GCL of the CC dorsomedially. The plexiform layer shows some prominence containing a few dispersed neurons while the LCpm is very prominent upon the dorsal surface of the foramen. Of particular note is the conical mass of displaced granule cells that appear to emanate from the LCpm, dorsolaterally in the direction of the granule cell layer of the LC.

FIGURE 3

This is a rostral section through the gymnotid brain demonstrating the continuity of cholinesterase staining between the eminentia granularis, zone χ and the caudal lobe. The corpus cerebelli remained unstained, typical of the divisions found between archicerebellum and the other cerebellar regions of many other vertebrates.

FIGURE 4

Pars medialis region of the caudal lobe showing the fine structure of the dorsal surface of the foramen, indicative of a pial surface by virtue of the flat non-ciliated cells that line the opening. Insert shows a low power magnification of the same opening where dorsally the foramen lining does not show the heavy stain of the ventral columnar epithelial lining. The latter is characteristic of ventricular wall linings (Peters et al., 1976).

FIGURE 5

Granule cells drawn from Golgi-impregnated material showing the short radiating dendritic processes and terminal claws that participate in the glomerular structure. Arising from the small round somata, and coursing through the granule cell layer towards the molecular lamina, are the thin granule cell axons. Occasionally these axons may be seen to bifurcate upon reaching the MOL.

FIGURE 6

EM. of granule cells in the granule cell layer. Characteristically, the nucleus occupies a great portion of a cytoplasm that is sparse in organelles. Originating from the granule cell soma is a thin dendritic process. Closely apposed to the central granule cell are adjacent granule neurons. In the bottom right hand corner is a glomerulus involving, quite typically, a central mossy fiber rosette making asymmetric contact with surrounding granule cell dendrite claws. Superimposed upon the glomerulus and making symmetric contacts with the granule cell dendrites is a terminal containing a pleomorphic vesicular population and believed to be a Golgi cell axon terminal.

FIGURE 7

Golgi-impregnated granule cells and mossy fibers in the granule cell layer of the caudal lobe. Note the large globular expansions of the mossy fiber as it courses through the granule cell lamina. The dense packing of the granule cells suggests the possibility of multiple involvement of these neurons in a single glomerular structure.

FIGURE 8

Schematic representation of a glomerulus drawn from an EM micrograph. This is a mossy fiber rosette of the dispersed variety containing small round and dense core vesicles as well as a number of coated vesicle types. The mossy fiber displays the complex interdigitation with dendritic claws, characteristic of glomeruli of most vertebrate cerebella. However, it is evident that two varieties of dendritic processes are present - one being more sparse in reticular matter than the other. Occasionally, in the more electron lucent dendrites, neurofilamentous clusters may be seen. Typical of most glomerular structures are the numerous desmosomal contacts between dendrites. The mossy fiber displays two types of junctional synapses with the dendritic claws - the conventional asymmetric synaptic contacts and the gap junctional synapses. On the periphery of the glomerulus, and contacting the dendritic claws, lie numerous small terminals, containing a pleomorphic vesicle population, that make symmetrical contacts. These are believed to be Golgi cell axon terminals. Surrounding the glomerulus is a glial envelope.

FIGURE 9-

Comparative ultrastructure of compacted and dispersed mossy fiber terminals. Although the terminology refers principally to the degree of compaction of the vesicle populations of each rosette, it usually follows that the mitochondria assume a parallel format. Both types of junctions can be seen between mossy fibers and the dendritic processes involved in the glomerulus. As well, the occasional dense core and coated vesicle can be seen.



FIGURE 10

A Golgi neuron deep within the granule cell layer of the caudal lobe showing thin radiating dendrites from a spherical and slightly rugose somata. Although the dendrites usually ramify locally, an occasional process can be seen to extend somewhat distal to the cell body. Axons are sinuous in nature, arising either from the cell body or proximal dendrites and tend to remain locally.

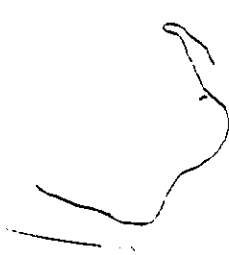


FIGURE 11

A Golgi neuron superficial to the granule cell layer exhibiting a form generally similar to the deeper varieties. More importantly, however, is the position of dendritic processes - most notably those that ramify within the adjacent plexiform and molecular laminae.

FIGURE 12

A) Some Golgi cell axons terminate upon the dendritic processes found in the glomerulus while

B) some project axons the full breadth of the granule cell layer to terminate upon dendrites within the plexiform and molecular layers.

FIGURE 13

A Golgi neuron in the deep GCL of the caudal lobe. The roughly spherical cell body has a rugose surface that occasionally shows contact by mossy fibers terminating in the granule cell layer. The cytoplasm has a density imparted by numerous free ribosomes and small mitochondria. A thin dendrite can be seen as it leaves the cell body.

FIGURE 14

Parallel fiber contacting a Golgi cell dendrite
in the plexiform layer of the caudal lobe. Quite
characteristic of these dendrites are the large axial
mitochondria and the electron dense nature of the
cytoplasm.

FIGURE 15

Stellate neuron drawn from Golgi-impregnated material. The tortuously ramifying dendrites contain numerous spicules and these processes appear to be confined to a region around the cell body.

FIGURE 16

A Purkinje neuron showing the orientation of the dendritic arborization. This is a sagittal orientation demonstrating that the branching pattern of the dendrites is more planar in the sagittal view. An axonal plexus is often viewed as it leaves the basilar pole of the Purkinje neuron to terminate locally within the plexiform layer of the caudal lobe. Sometimes the axonal processes cross the somata of the Purkinje cell.



FIGURE 17

A Purkinje neuron in the molecular layer of the caudal lobe adjacent to the plexiform lamina. A large cell body that is slightly rugose is enveloped in a thin glial sheath. The cytoplasm is electron dense and is comprised of numerous ribosomes and mitochondria arranged in a "whorl-like" fashion around a large nucleus. This Purkinje neuron is closely opposed to an adjacent Purkinje cell and shows a portion of a climbing fiber terminal making contact on its somatic surface.

FIGURE 18

Recurrent collateral from a Purkinje cell axon making contact with the axon hillock and initial segment of a eurydendroid neuron located in the plexiform lamina. The axon loses its myelin sheath to a narrow collateral branch that opens up into a terminal containing a pleomorphic vesicle population. The junctional synapse upon the initial segment is symmetrical in nature. The axon hillock of this eurydendroid (pl) shows a slight amount of dense undercoating, an organized orientation of the micro-tubules and microtubular fasciculation - all of which are common features of axon hillocks of most neurons.

FIGURE 19

Electron micrographs of a parallel fiber and three climbing fibers showing comparative sizes and, in particular, the nature of the vesicular populations in each terminal.

a) The parallel fiber terminal contains a few small round vesicles and makes an asymmetric contact on the surface of a Purkinje dendritic spine.

b) The climbing fiber contacts the surface of Purkinje spines and often the smooth portions of their dendritic processes. Most prominent are the large dense core vesicles.

c) A climbing fiber terminal showing numerous dense core vesicles.

d) two large climbing fibers contacting numerous Purkinje spines in the molecular layer.

FIGURE 20

Comparative aspects of parallel and climbing fibers drawn in the molecular layer of the caudal lobe. The cf terminal is 3-4 times larger than boutons of parallel fibers, a feature that established an important criterion for distinguishing between these terminals at the EM level.

FIGURE 21

Richardson stained sections of the region of the plexiform lamina showing the comparative differences between Purkinje and eurydendroid (pl) neurons. The most prominent difference is the densities of their respective cytoplasmic matrix, the eurydendroid (pl) being considerably lighter than the Purkinje cell. While the Purkinje neuron is generally oriented perpendicular to the long axis of the plexiform lamina, the long axis of the eurydendroid (pl) cell runs parallel to the PFL.

FIGURE 22

EM of Purkinje and eurydendroid (pl) neurons in close apposition within the plexiform lamina. The cytoplasmic density imparted by the ribosomes and RER of the Purkinje cell is distinctly greater than that of the eurydendroid (pl).

FIGURE 23

Eurydendroid (pl) of the lateral caudal lobe showing the irregular nature of the somatic surface and the typical arrangement of the dendritic tree. Occasionally, the dendritic processes of both medial and lateral eurydendroid cells display an enlargement that can be described as an excrescence of the dendritic branch (INSERT). Spines are only occasionally seen upon the dendrites of these neurons.

FIGURE 24

Eurydendroid (p1) cell of the medial GCL showing a smoother cell body. Note how the dendrites ramify within the PFL, MOL and GCL and how, in sagittal sections (INSERT), the eurydendroid (p1) dendritic tree branches almost the full rostrocaudal layer of the caudal lobe.

FIGURE 25

A eurydendroid (pl) cell in the medial portion of the plexiform layer. Surrounded by numerous symmetrically contacting pleomorphic terminals, the neuron displays the sparsely arranged organelle constituents that are characteristic of eurydendroid cells. A few terminals are seen upon the somatic spine that originates from the cell body.

FIGURE 26

Purkinje terminals on eurydendroid (p1) cells including two terminals upon a somatic spine of a more lateral neuron. Note how the spine contains some cisternal material and a dense core vesicle. Often more than one contact site is made between the Purkinje terminal and the cell body of the eurydendroid (p1) cell.

FIGURE 27

Eurydendroid dendrites of the plexiform and molecular layers receiving a) parallel fiber contact and, b) climbing fiber contact. Note the presence of neurofilaments within the smooth eurydendroid dendrites and the large number of dense core vesicles contained within the CF terminal.

FIGURE 28:

Tract of the stratum fibrosum fibers entering the ELLL (lateral segment) sending collaterals dorsally to terminate as parallel fibers in the overlying molecular layer of the caudal lobe.



FIGURE 29

Schematic representation comparing the afferent circuitries of the mammalian and gymnotid fiber systems. The dotted line in d) indicates the one aberrant pathway of the gymnotid circuitry involving a direct granule cell - eurydendroid cell contact.

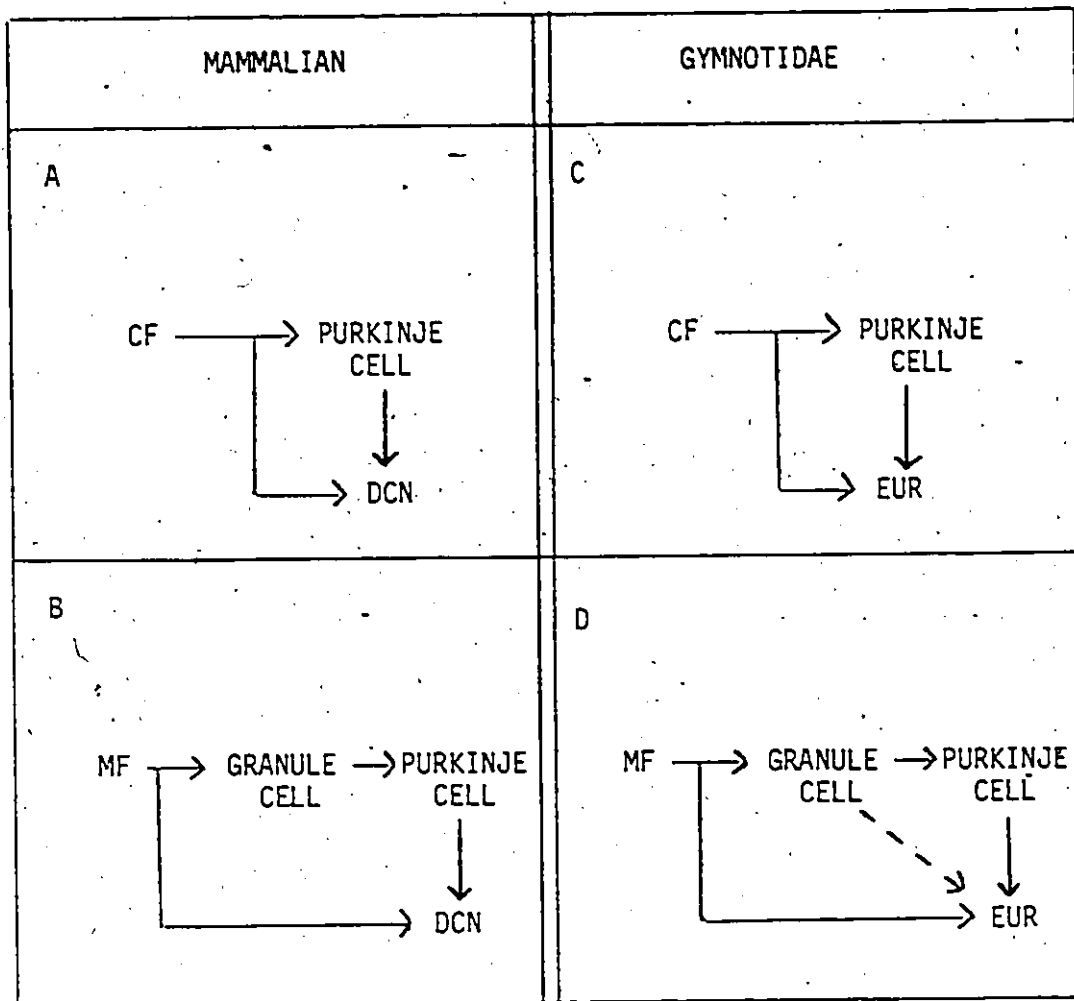


FIGURE 30

Summary diagram showing the synaptic relations of the afferent fiber systems and neuronal elements of the cerebellar caudal lobe of weakly electric fish and in particular the synaptological relations involving the eurydendroid (pl) cell.

Colour code:

- | | |
|---------------|---------------------------------|
| red | - eurydendroid neurons |
| blue | - Purkinje neurons |
| orange | - granule neurons |
| black | - Golgi neurons |
| yellow | - stellate neurons |
| purple | - mossy fibers |
| black (axons) | - tract of the stratum fibrosum |
| green | - climbing fibers |

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PLI

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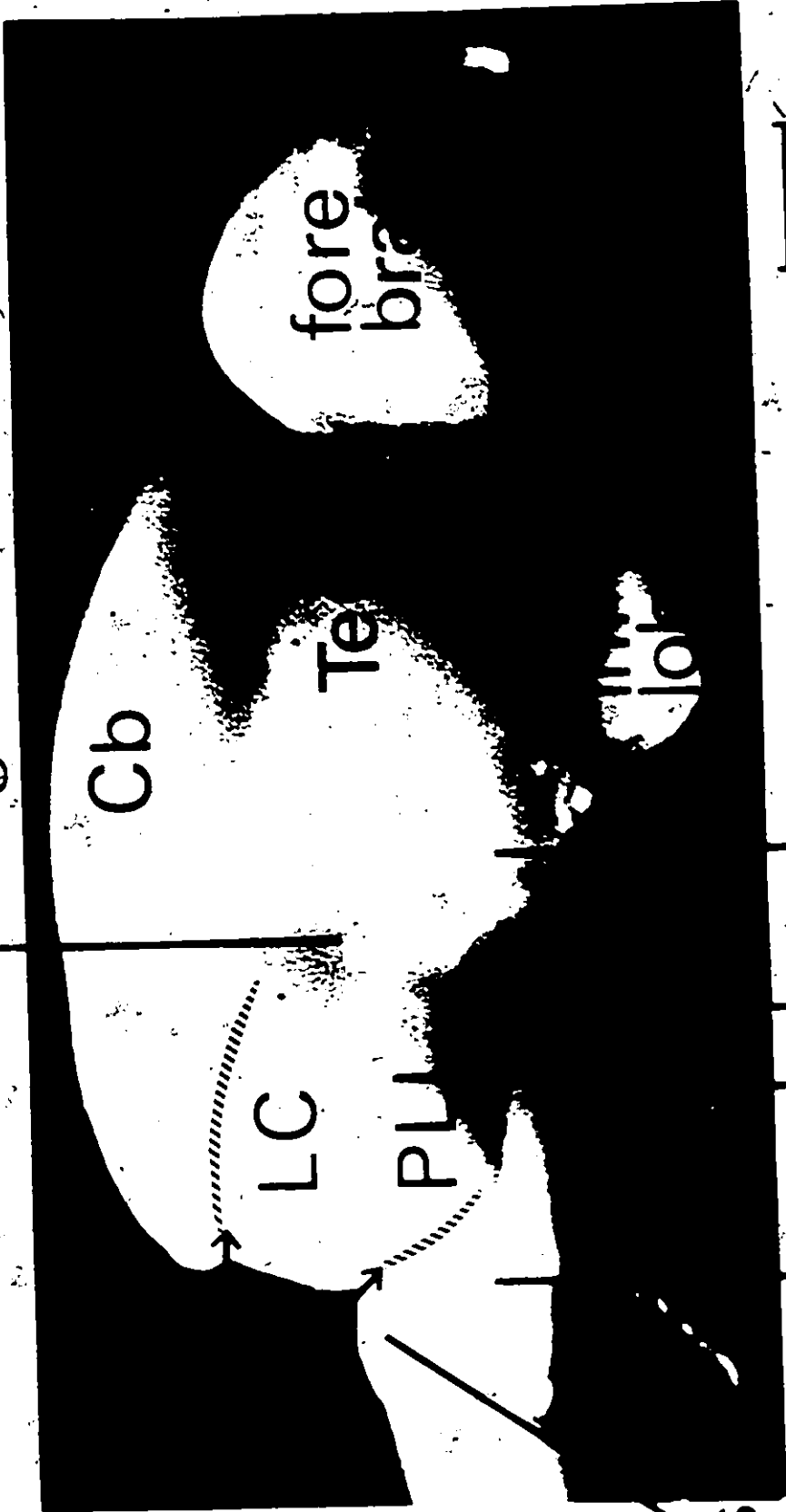
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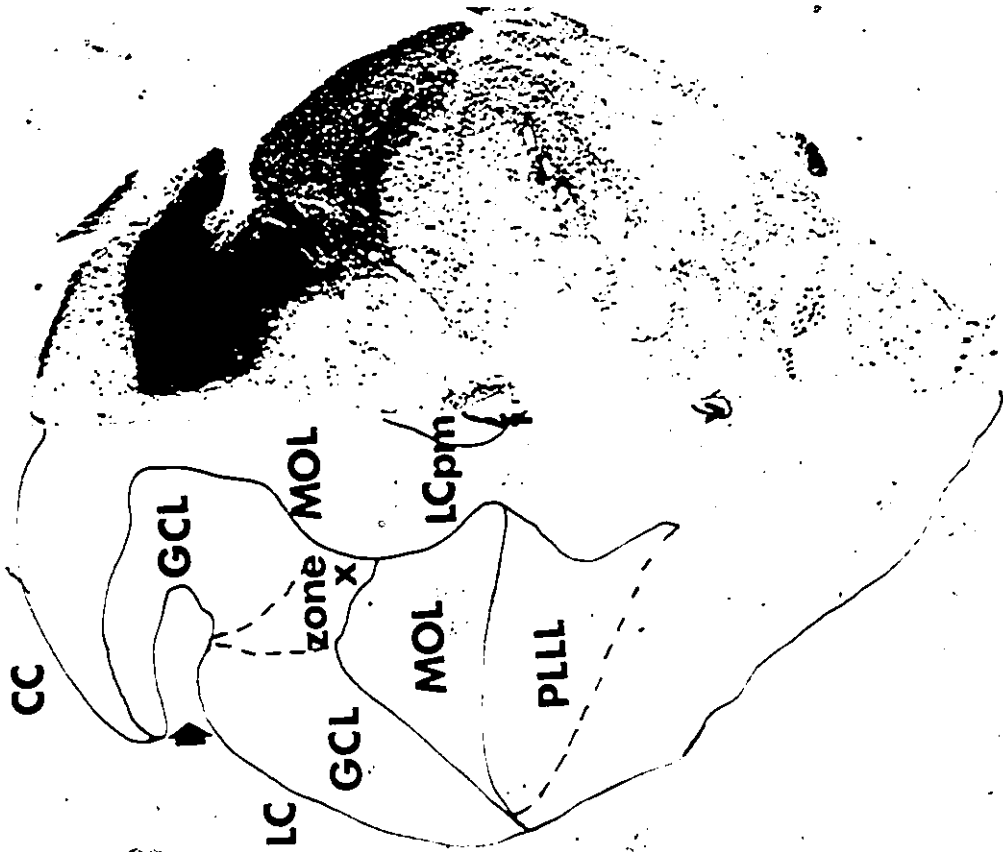
nXs

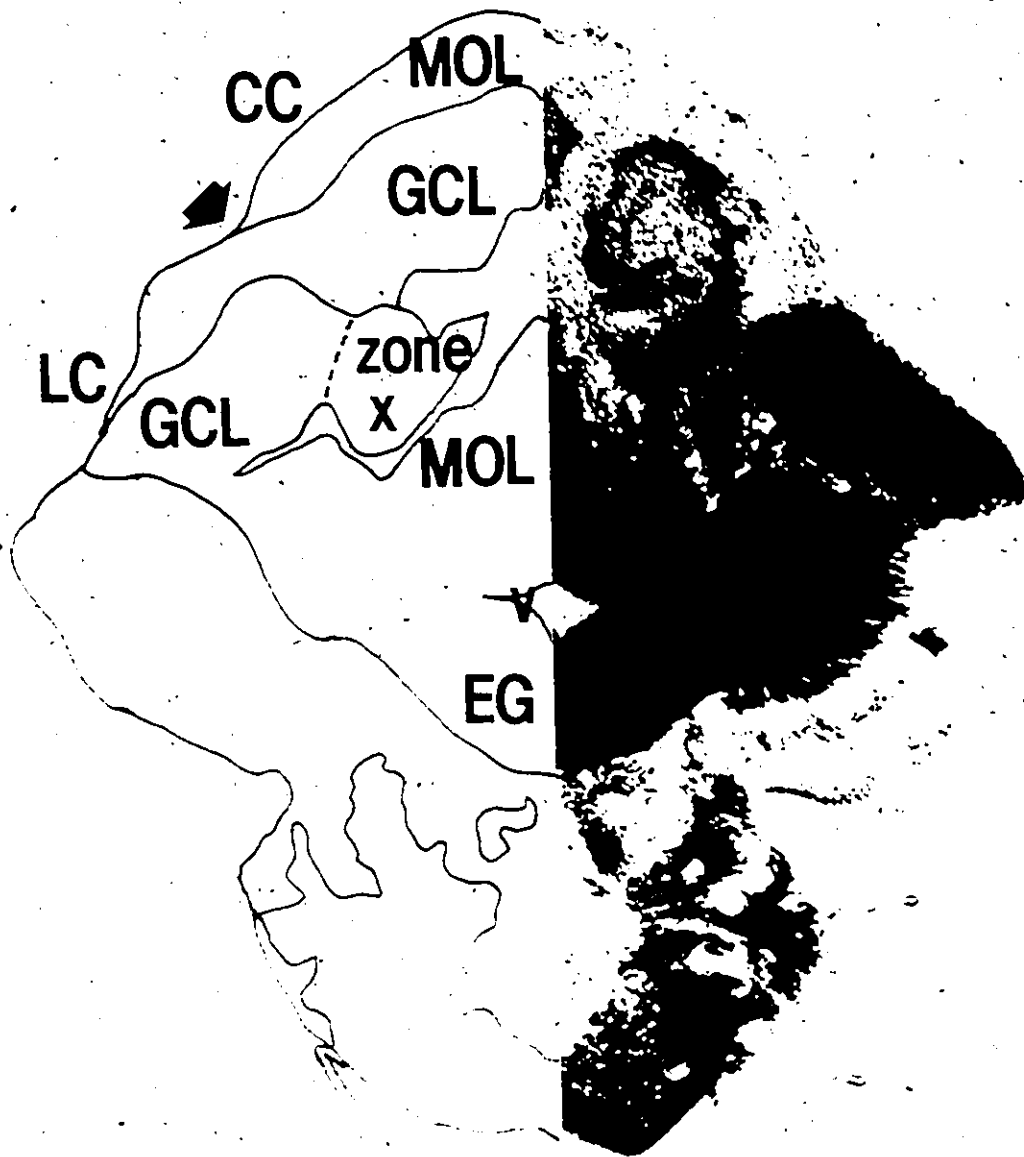
NLLp

MR nPr

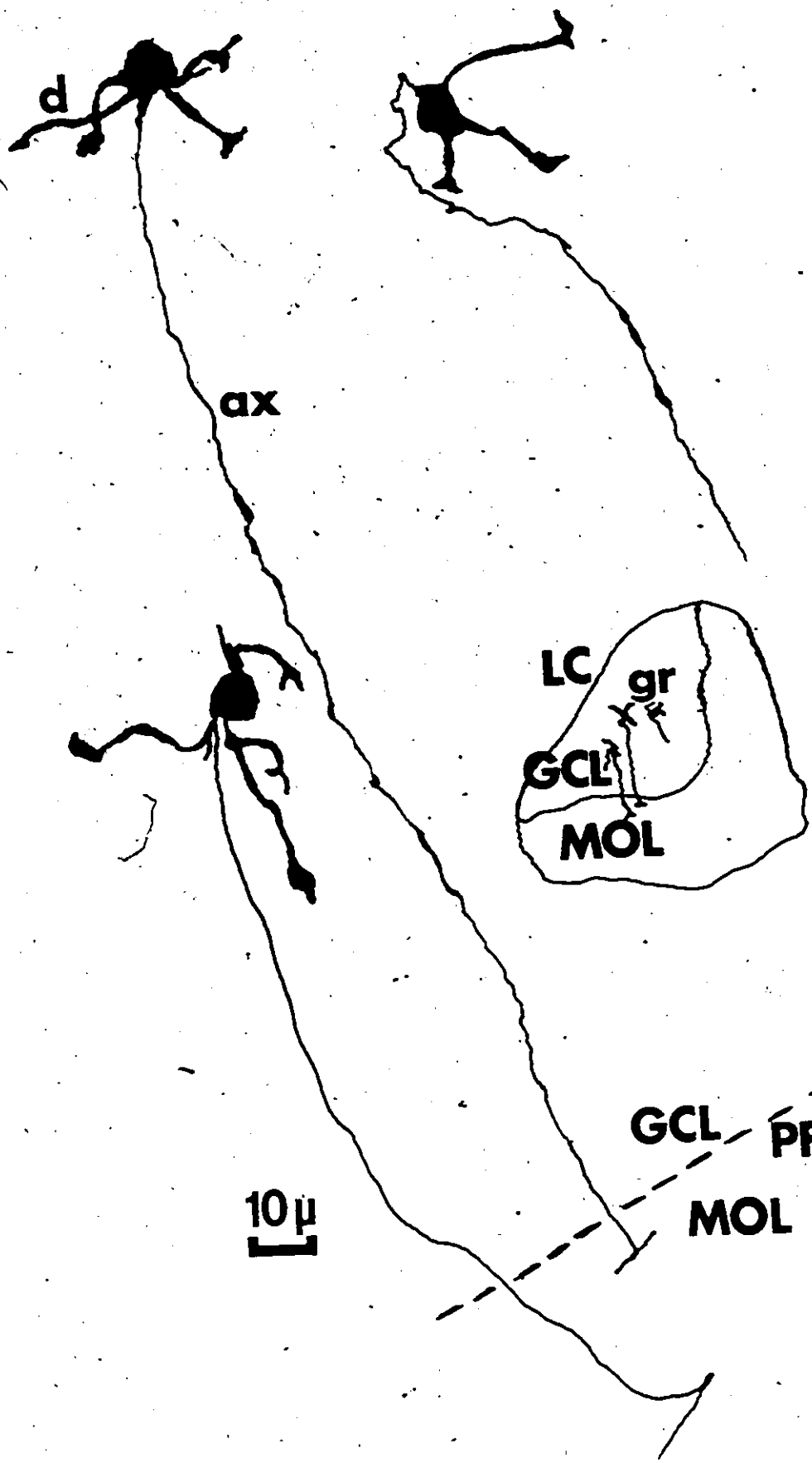
NLLa













1μ

d

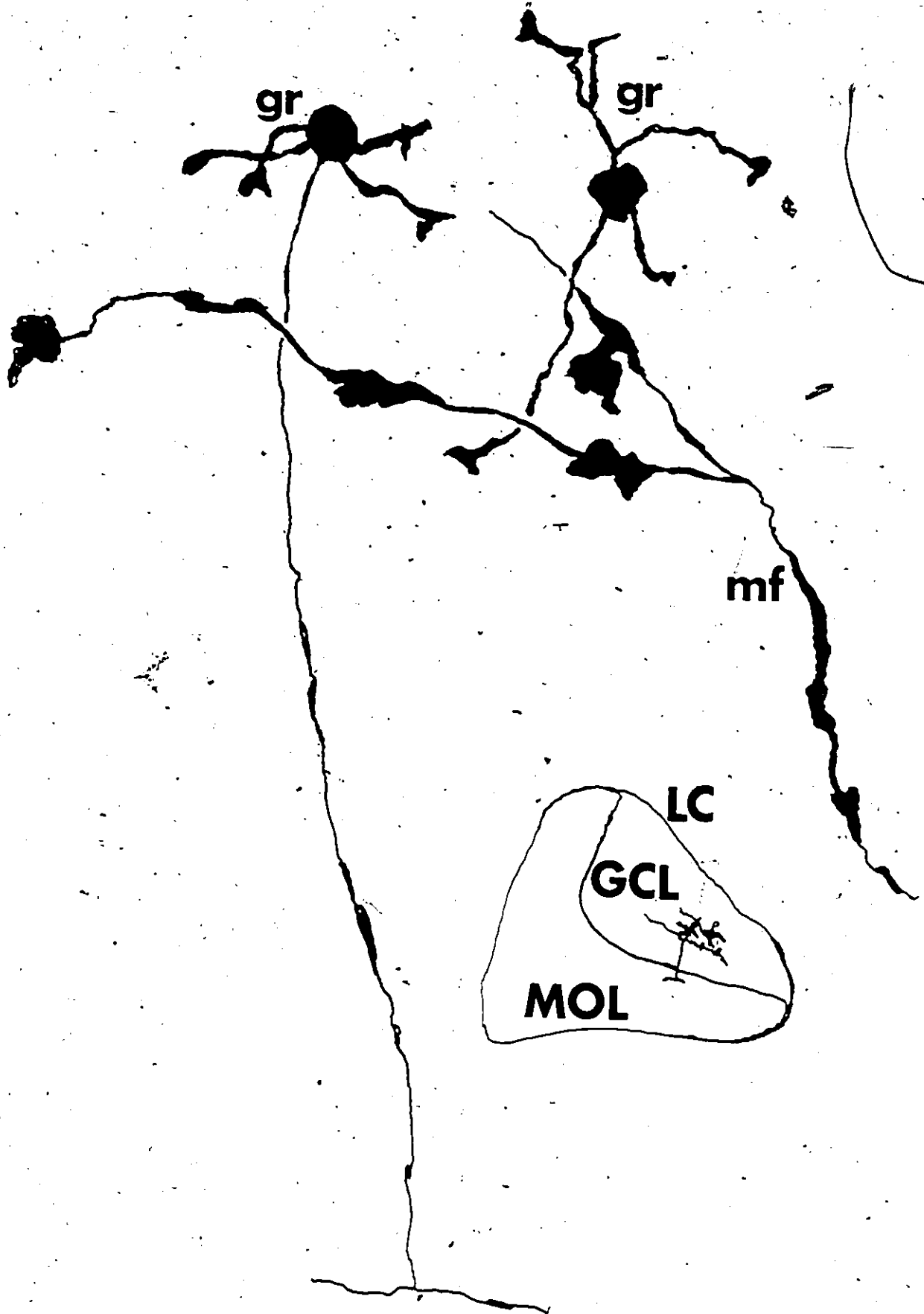
Gol

mt(d)

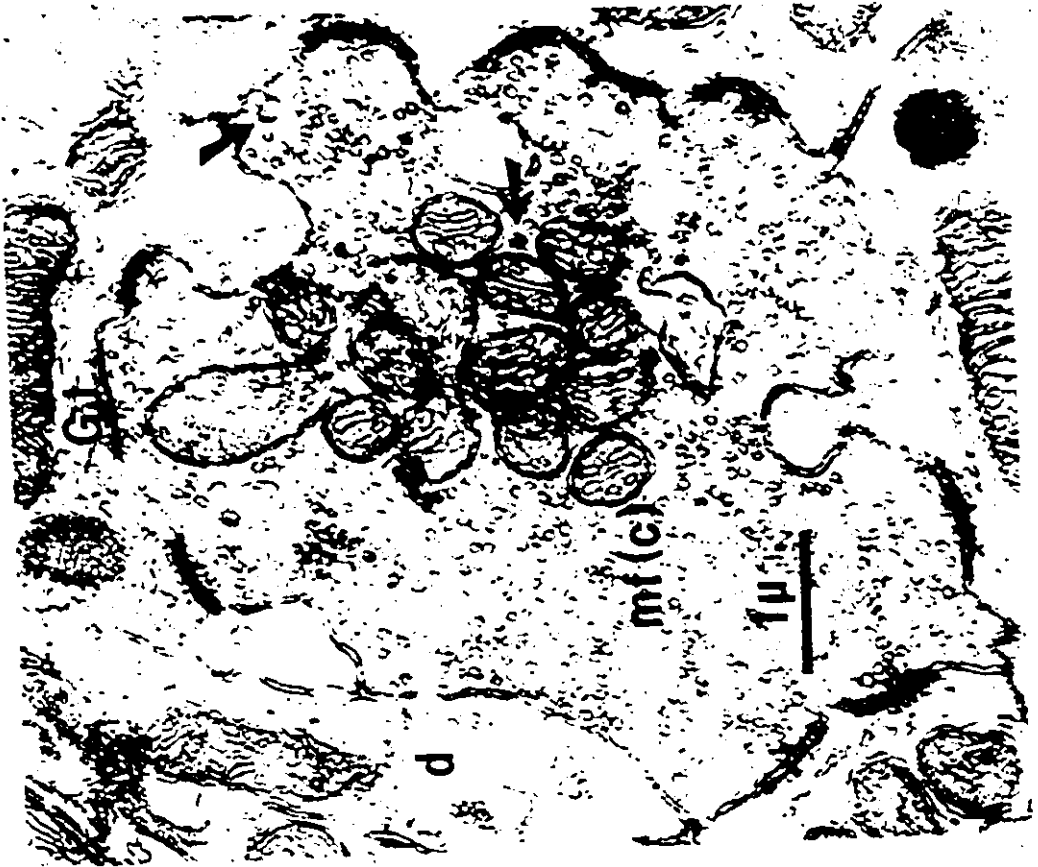
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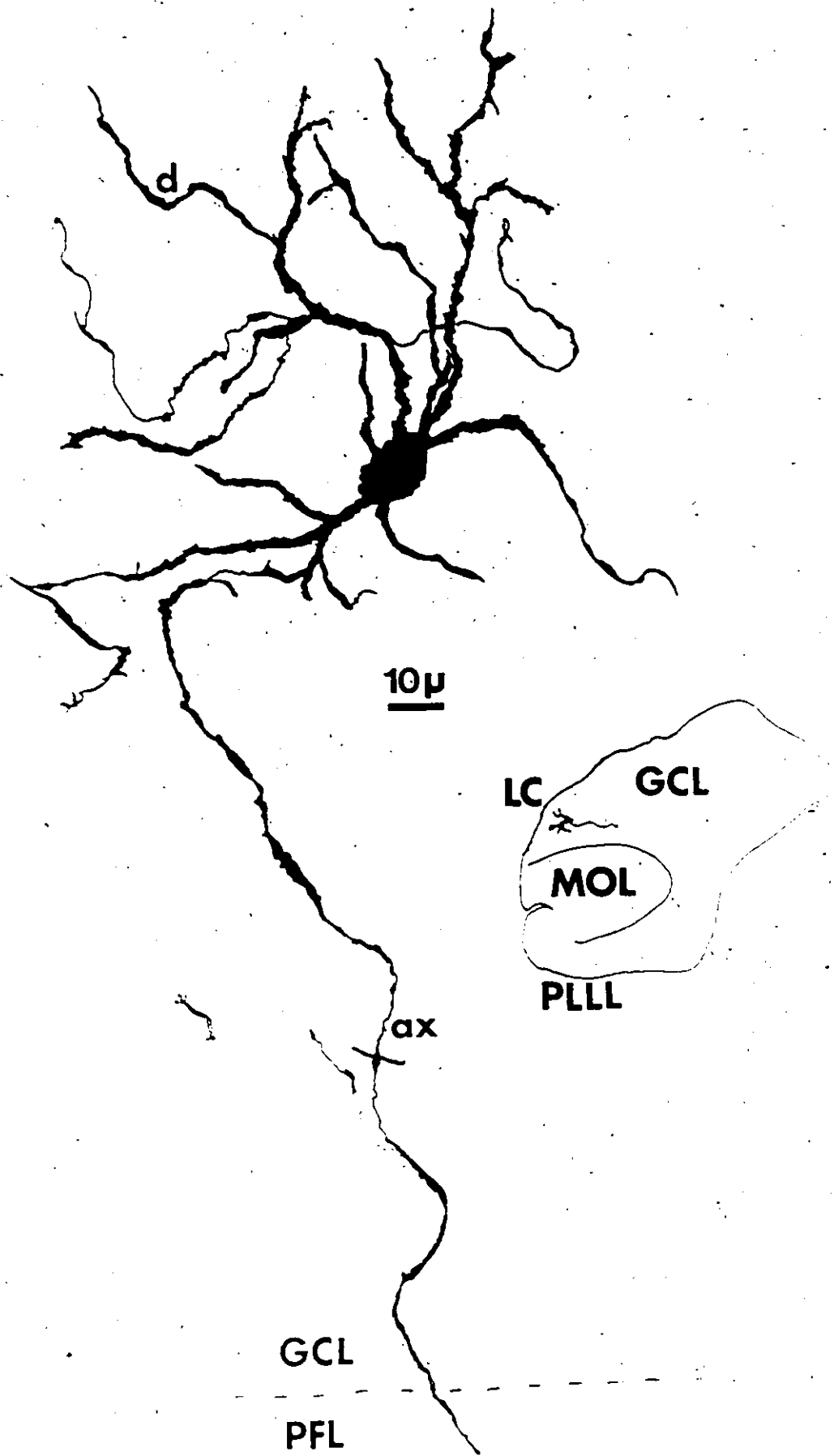
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GCL

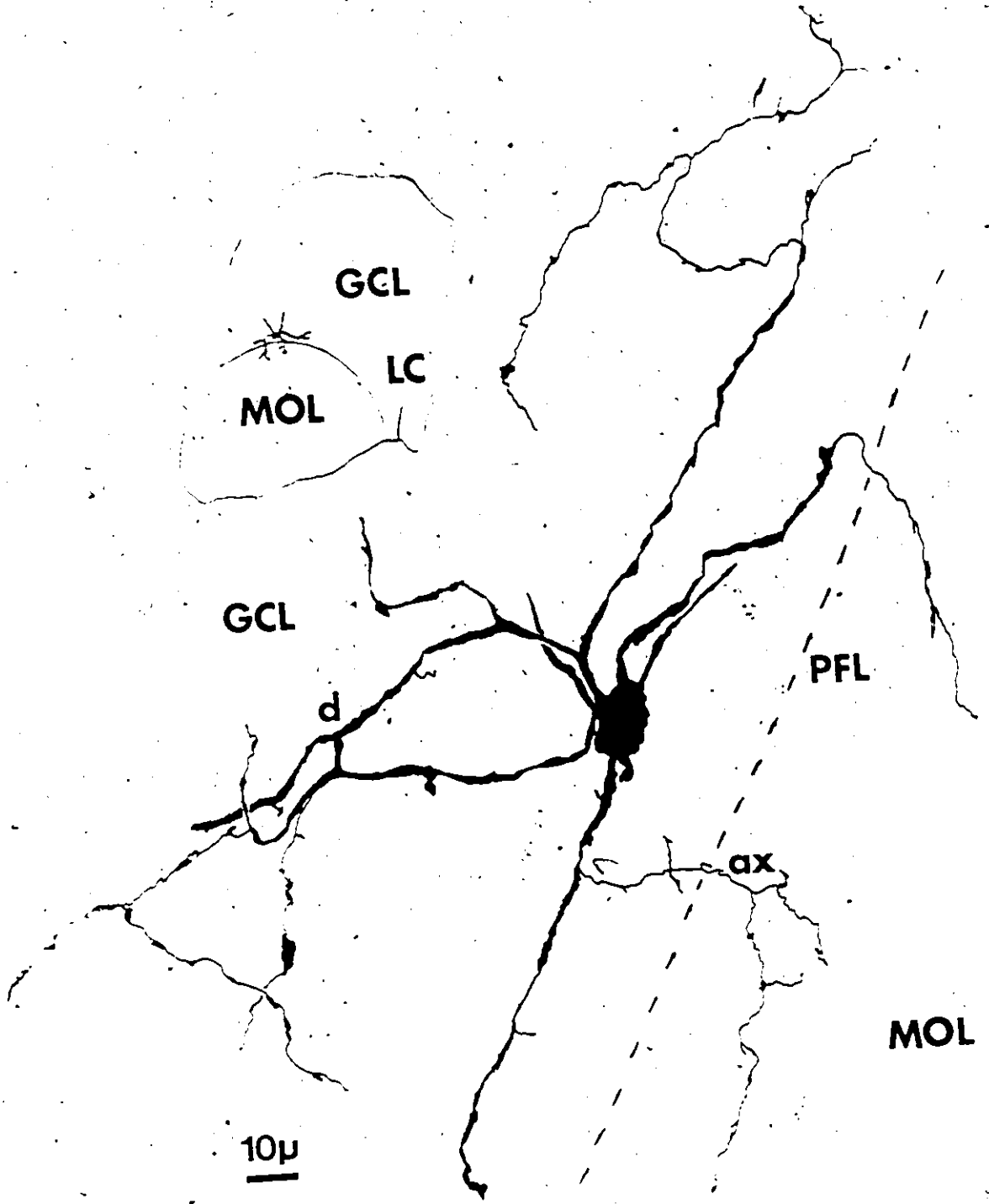
MOL

PLLL

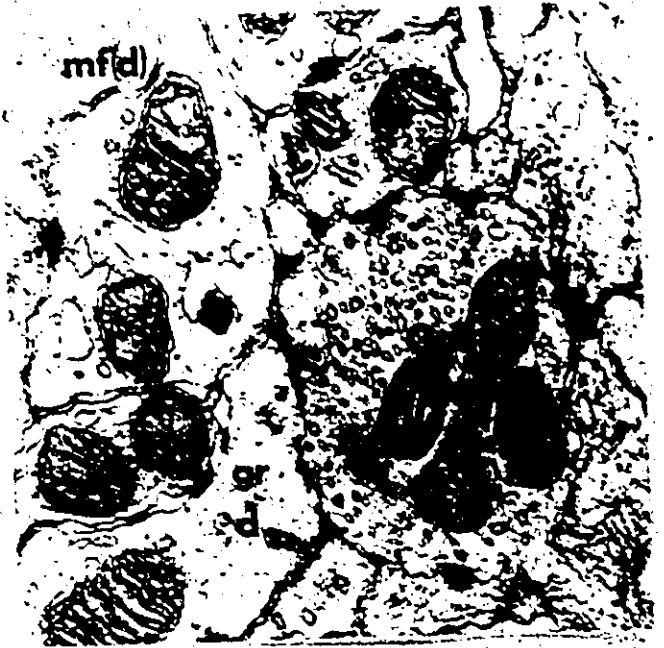
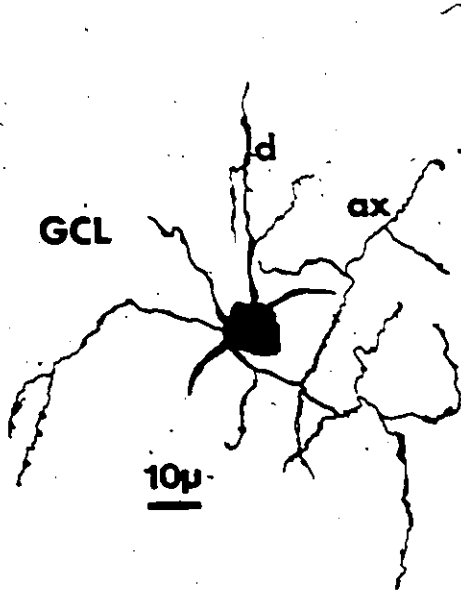
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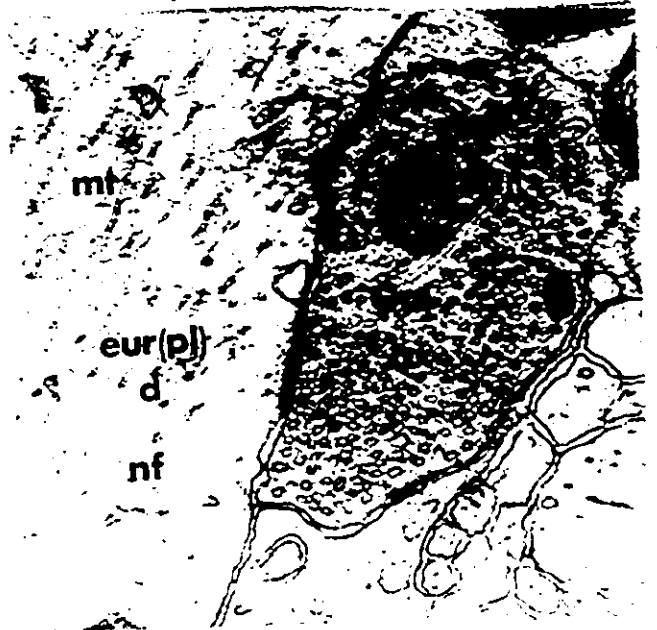
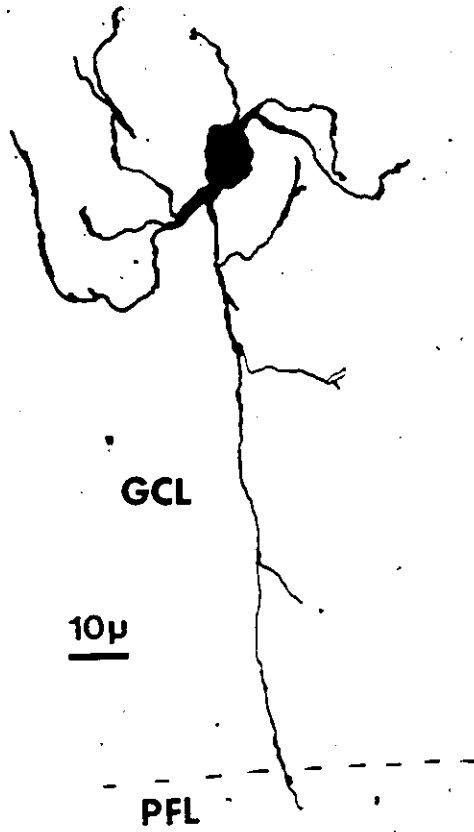
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A)

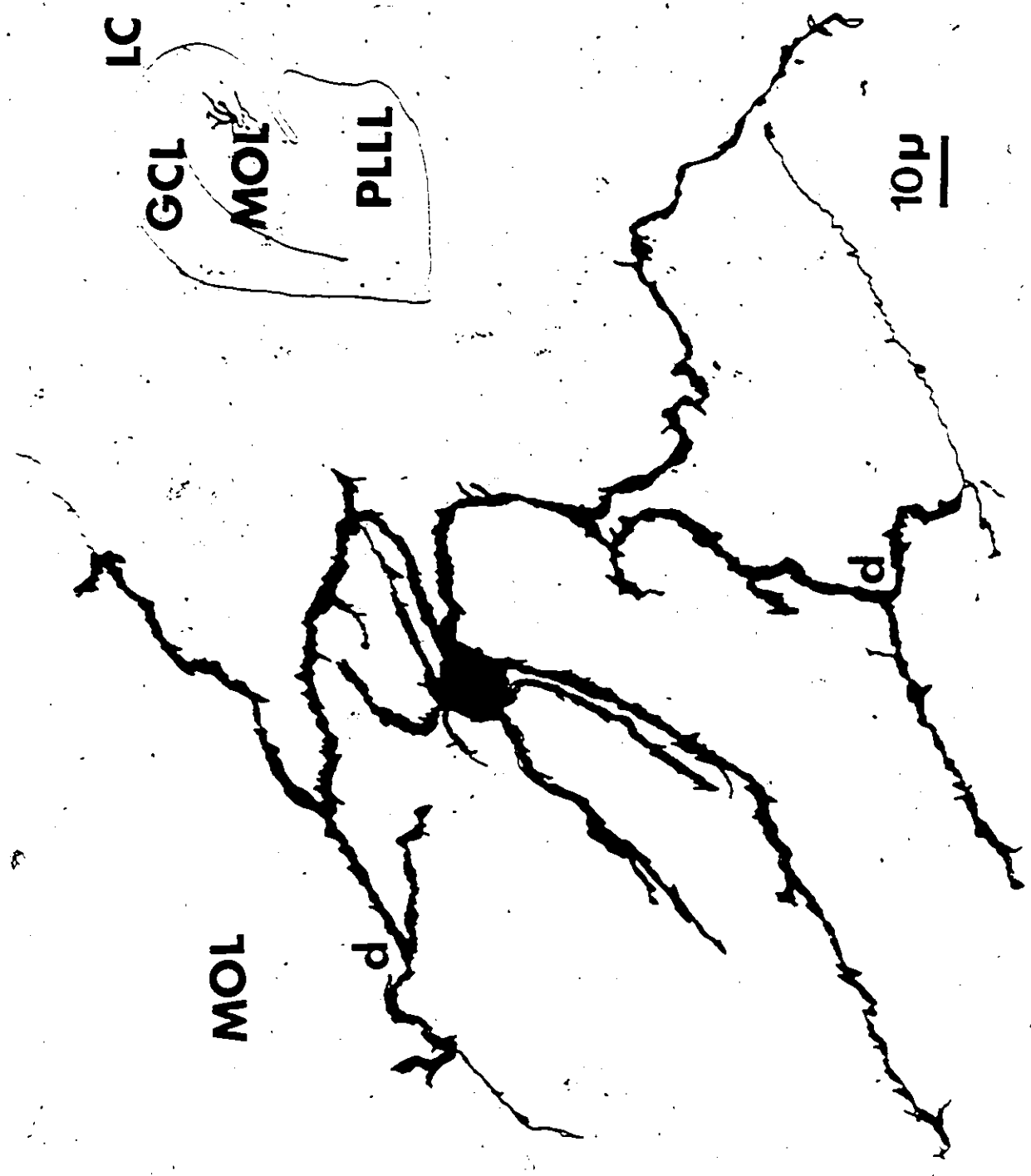


B)









LC

GCL

MOL

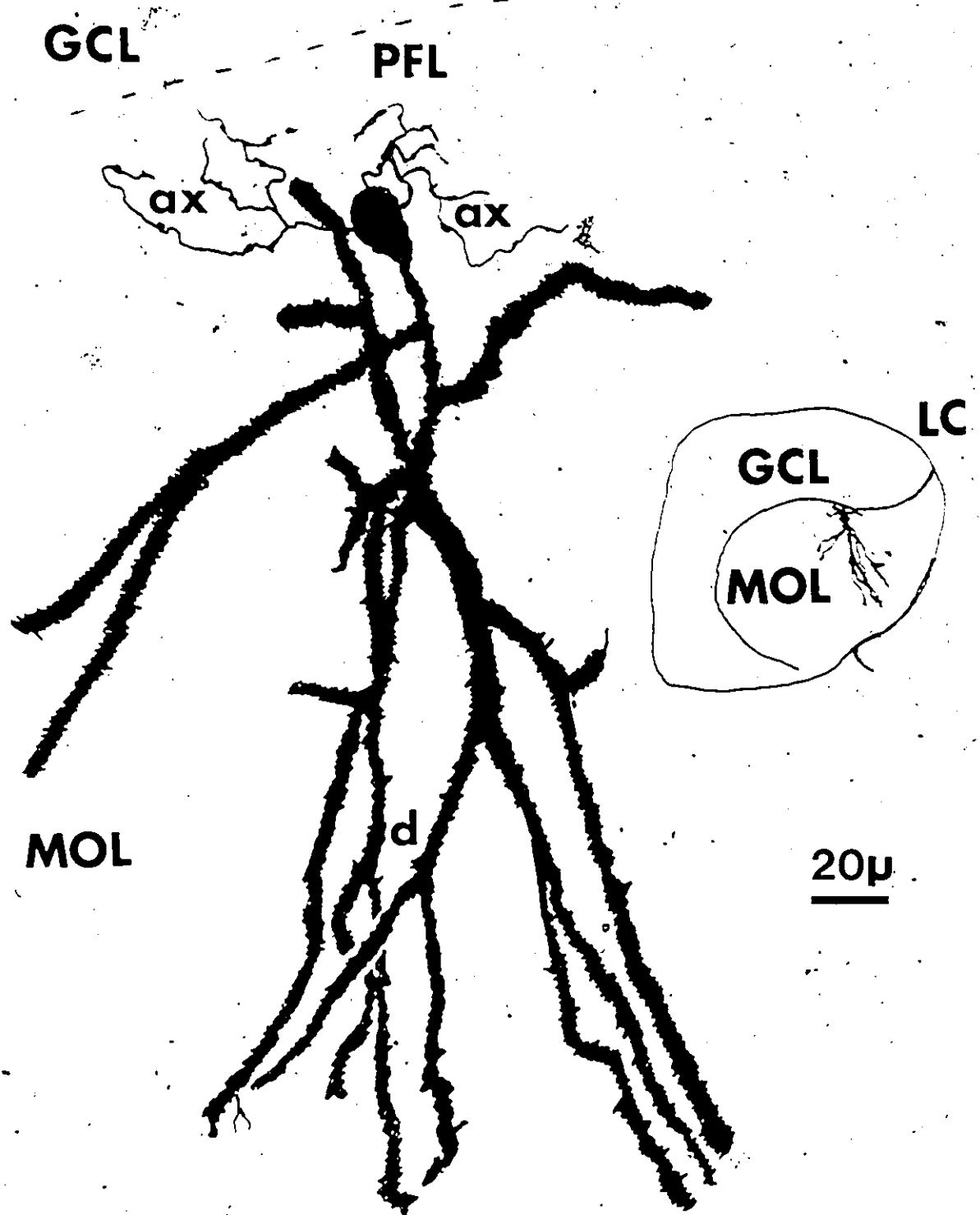
PLLL

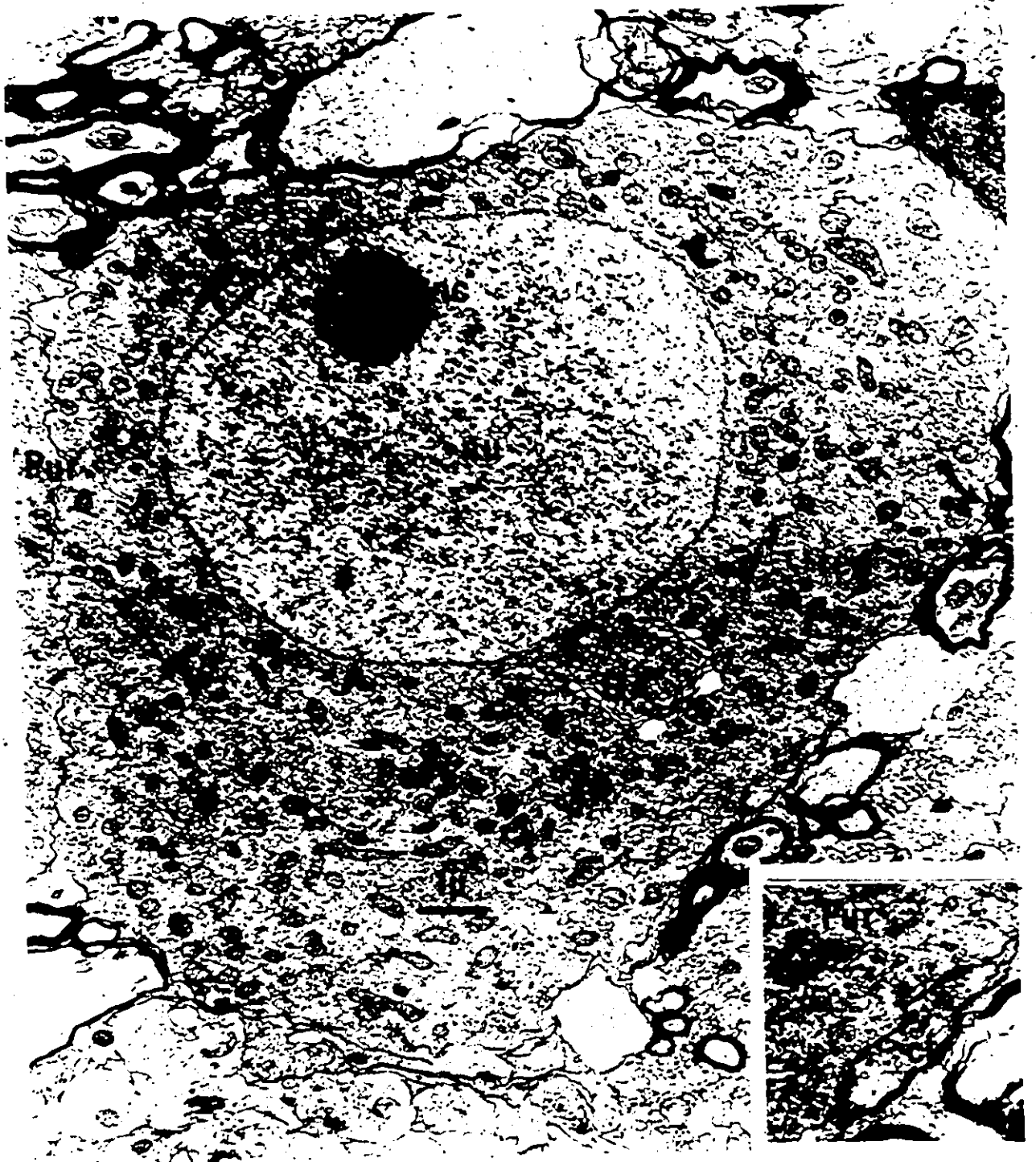
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MOL

d

d









B)



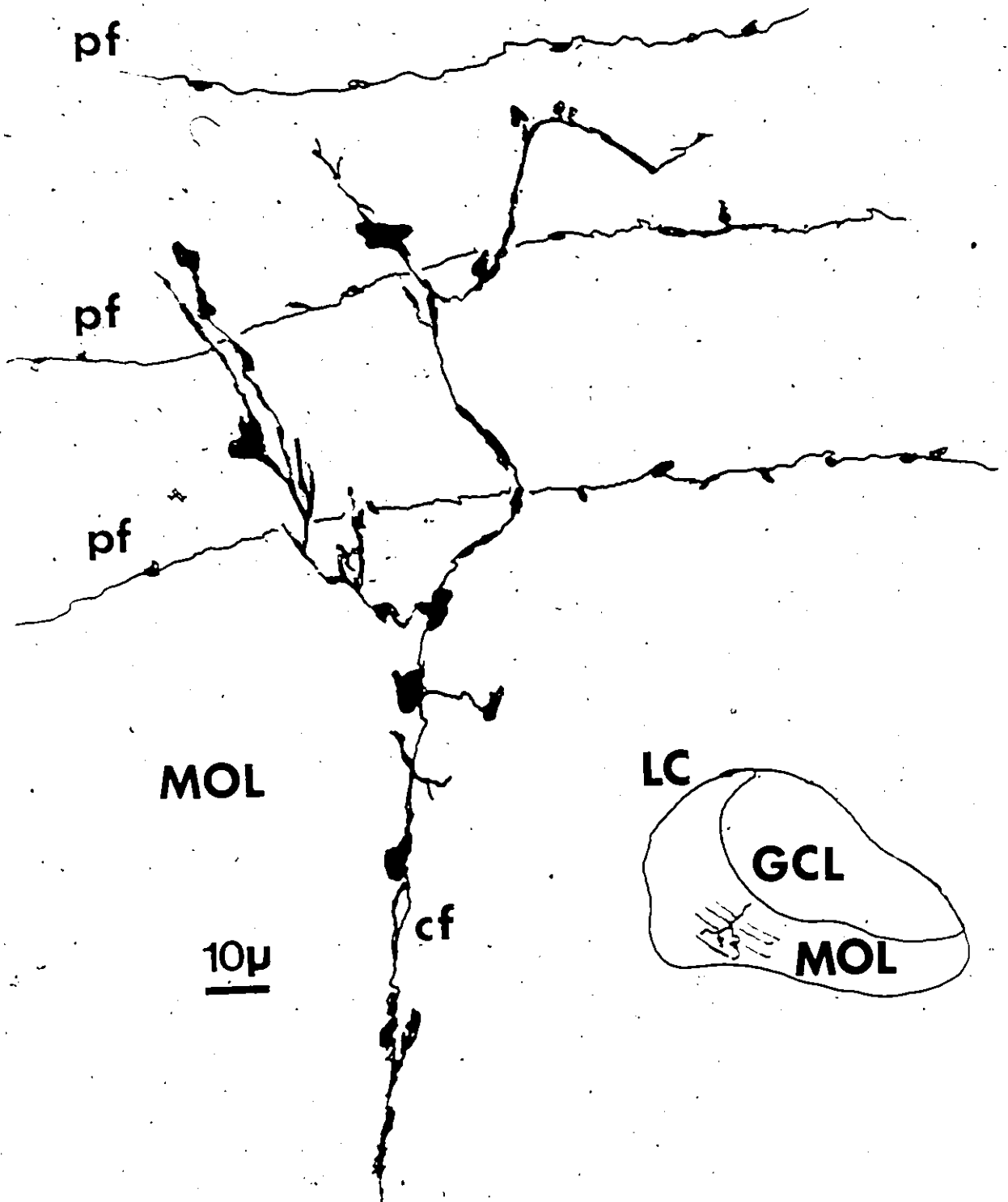
D)



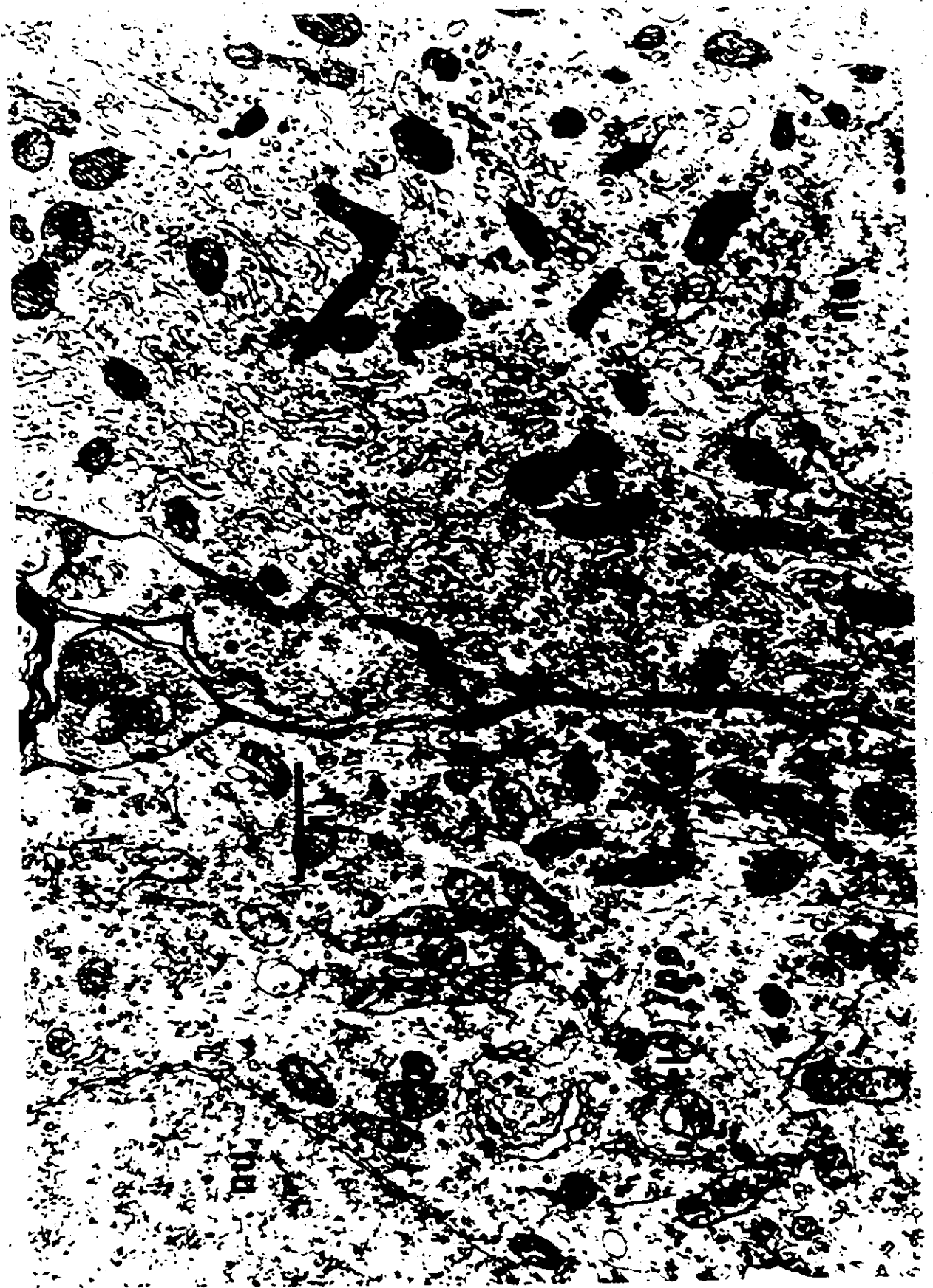
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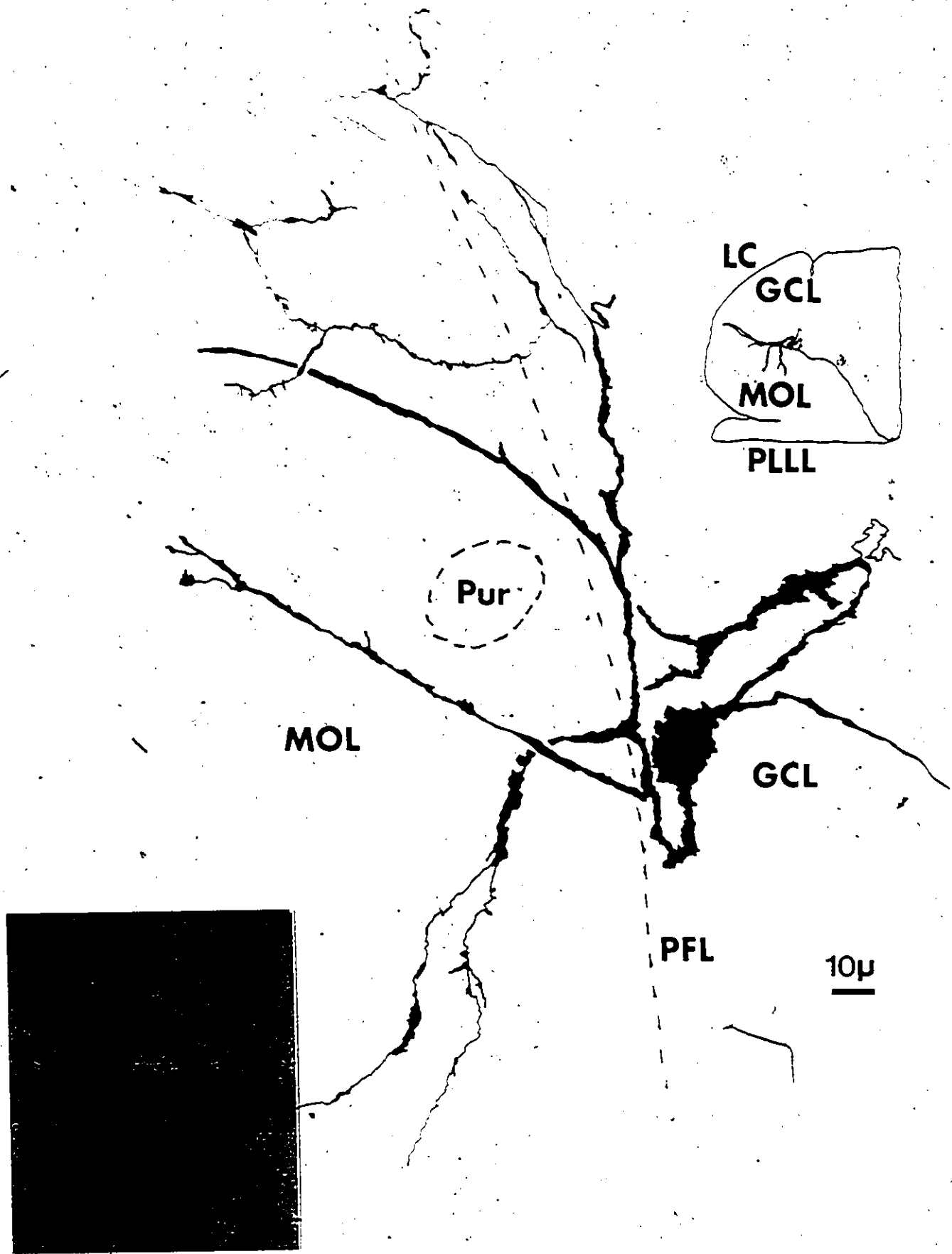


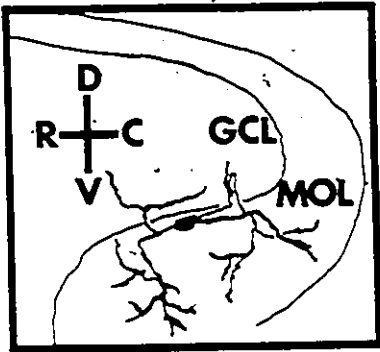
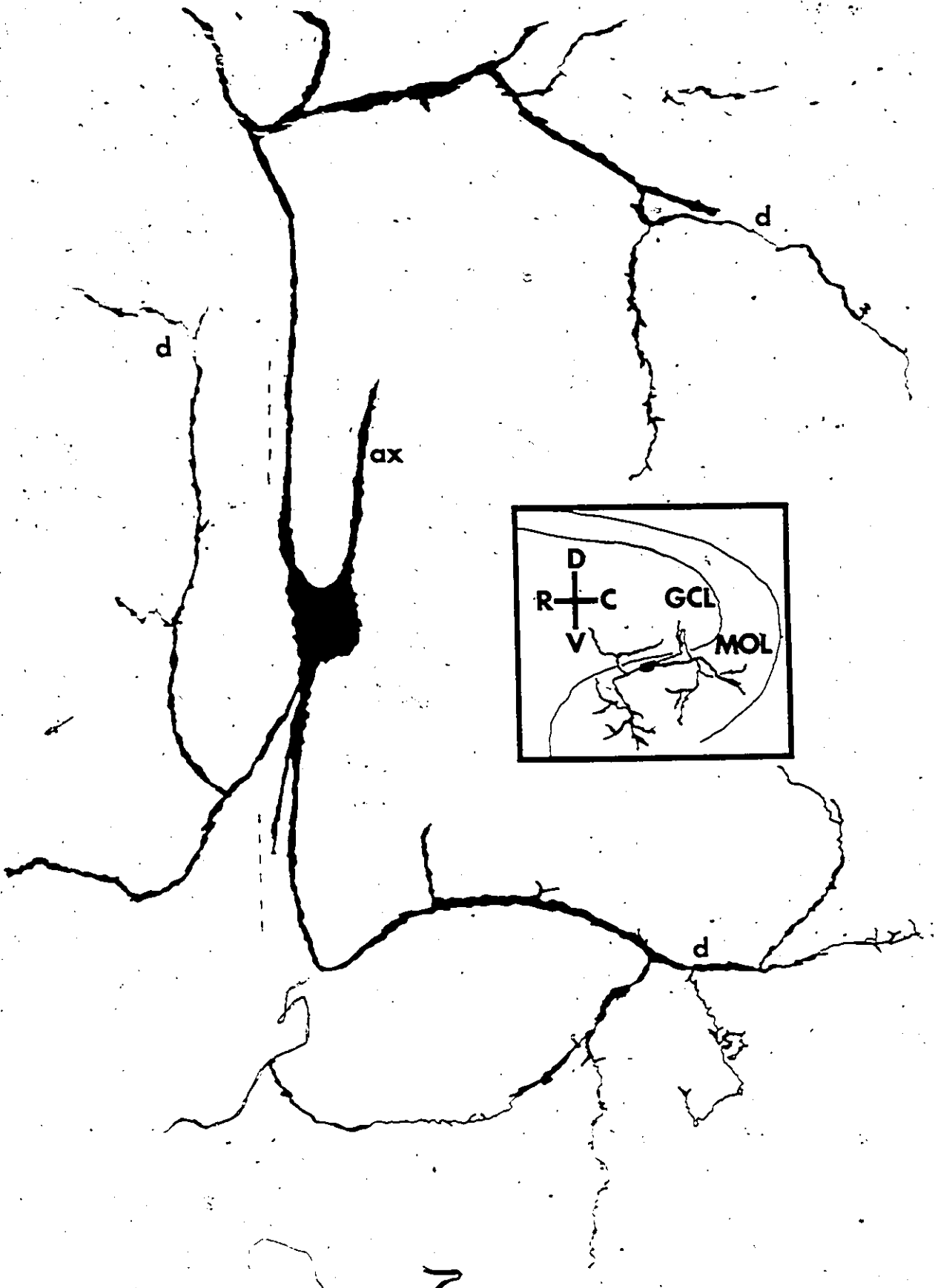
C)

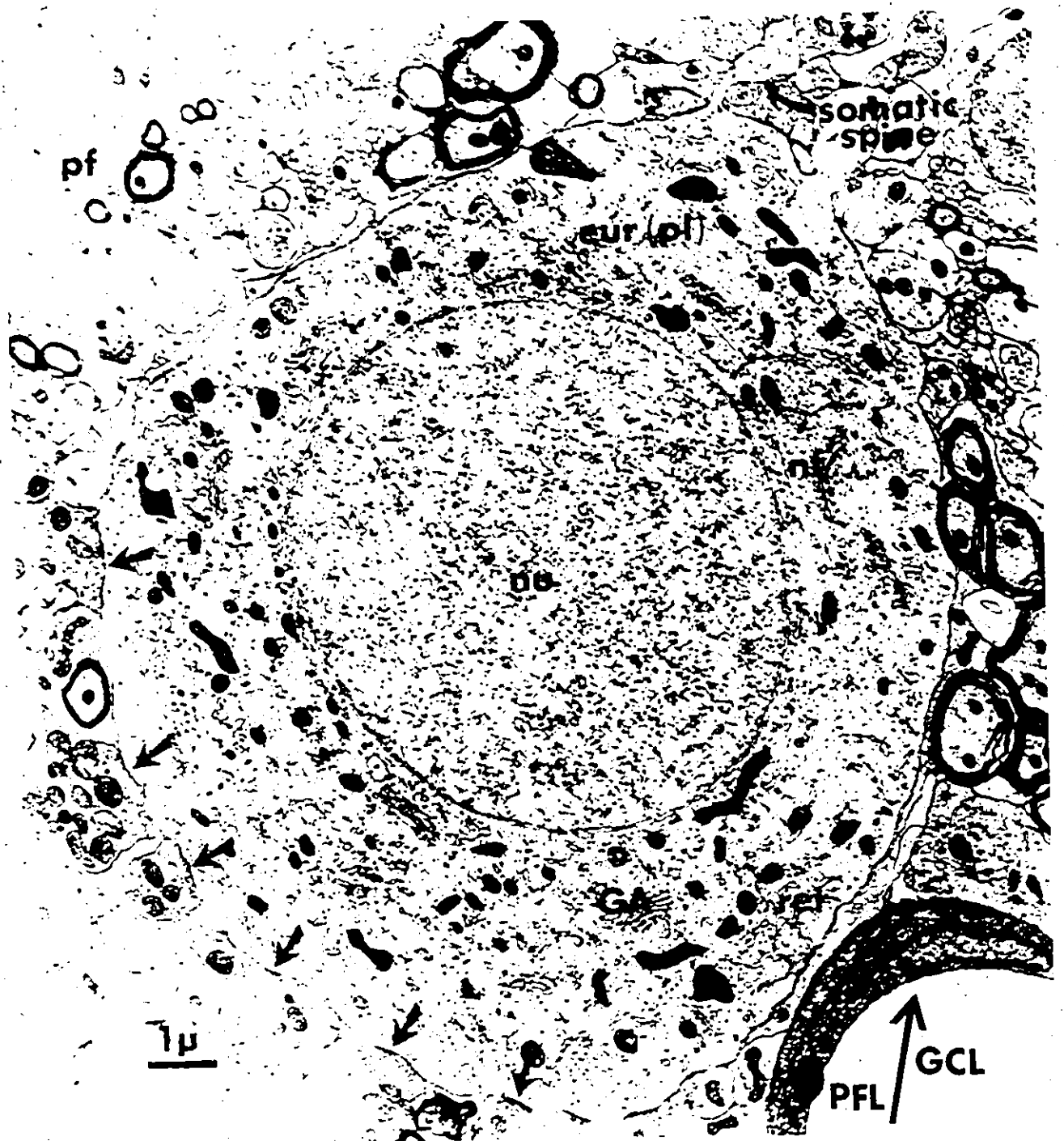




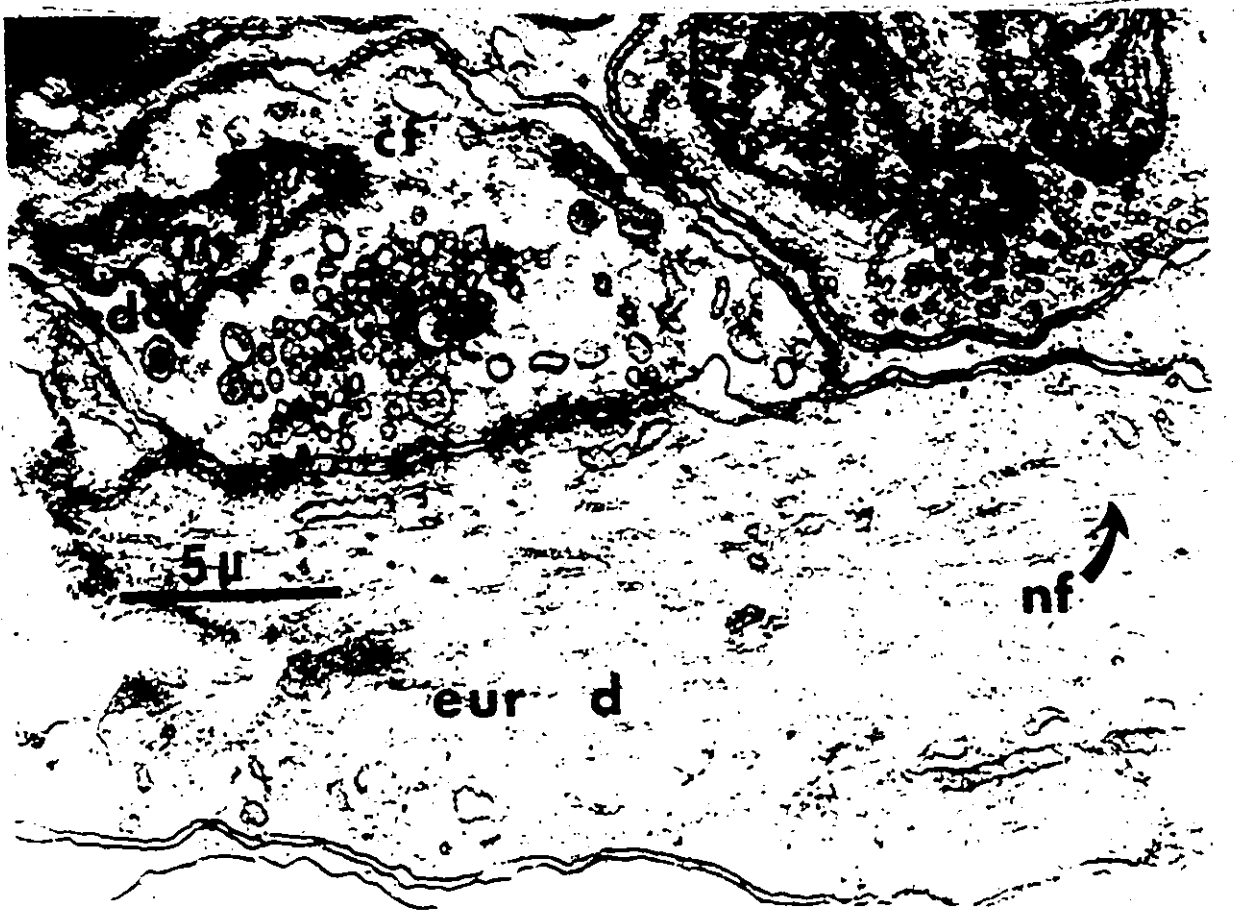
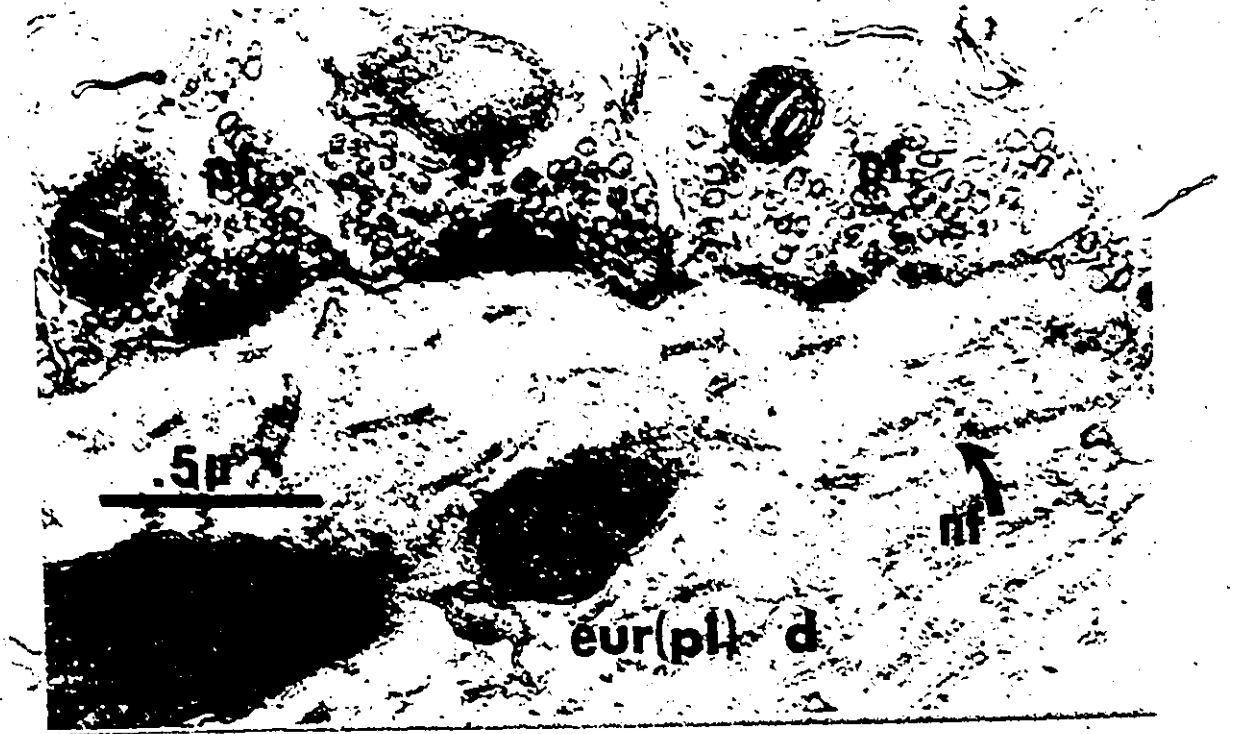


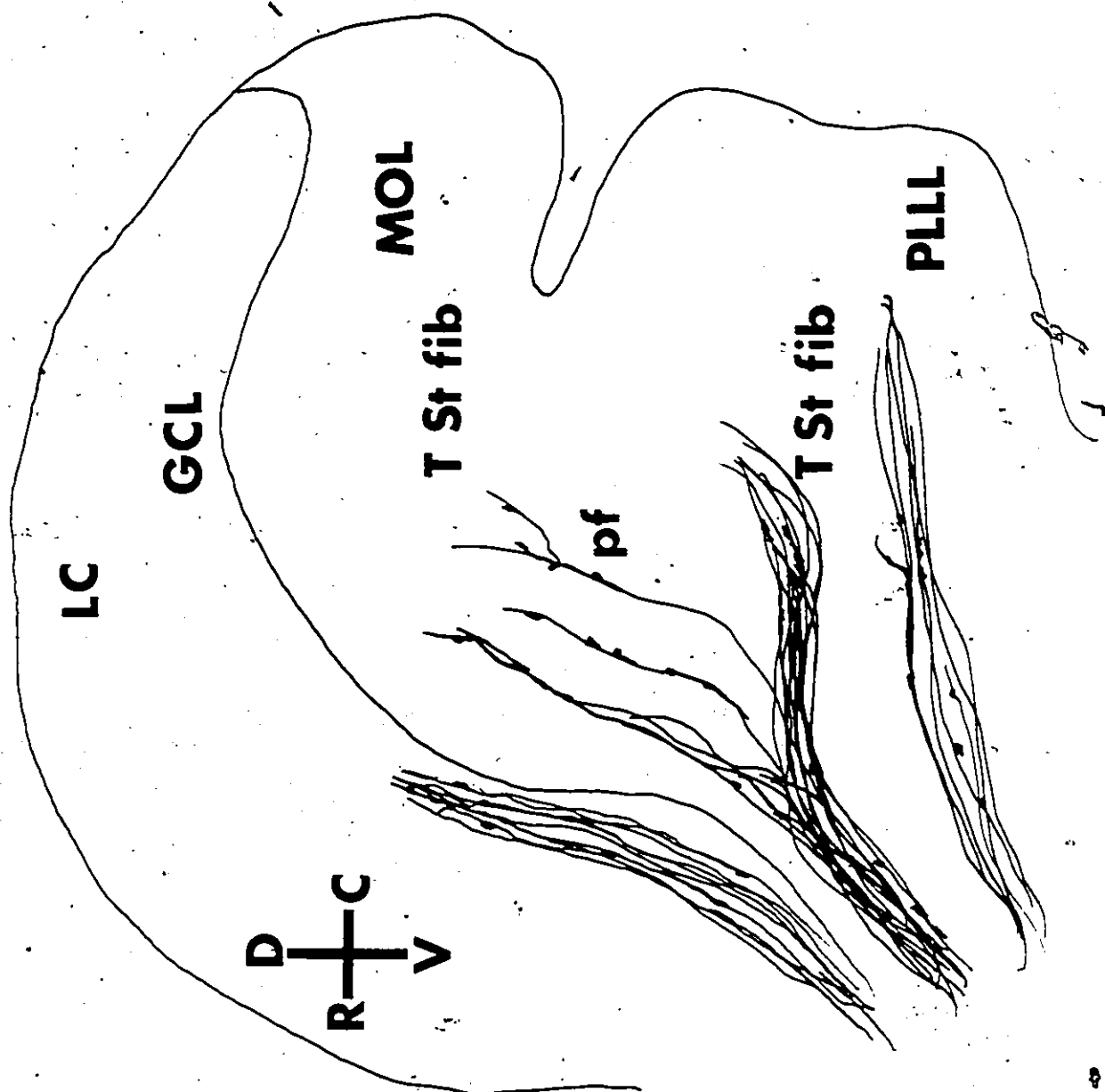


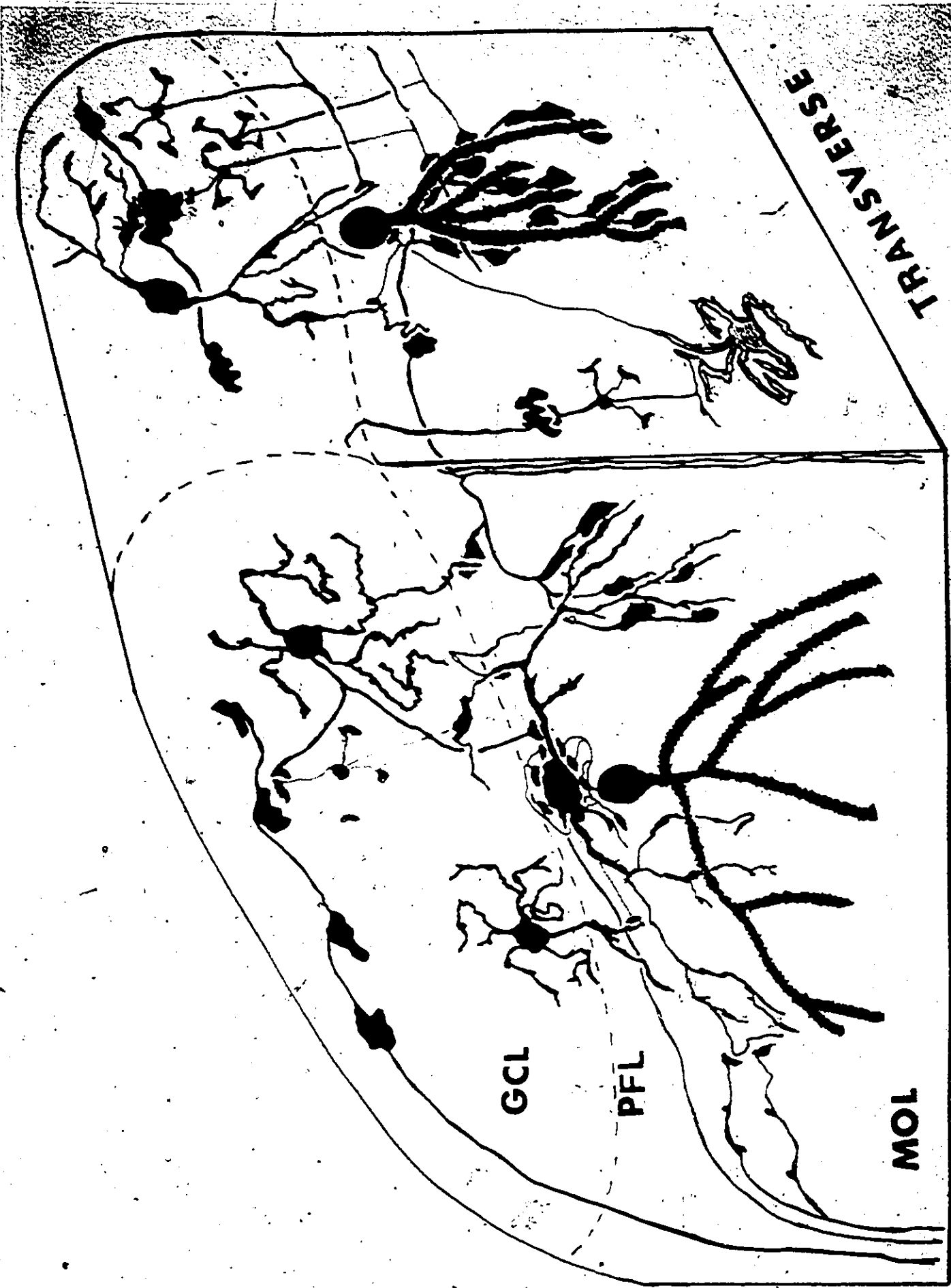












TRANSVERSE

SAGITTAL

GCL

PFL

MOL