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AFFERENT CONNECTIONS OF THE MAGNOCELLULAR NUCLEI OF THE HYPOTHALAMUS

by

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Dedication

To Mrs. J. Auer - an inspiring lady and a true friend.

To Dr. J. Auer, Professor of Anatomy - an inspiring scientist, admirable teacher, true friend and counsellor who, with profound patience and daily encouragement, has introduced me to the noblest of fields, research.

acknowledgment

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To them, this thesis is fittingly dedicated with the deepest sense of admiration and gratitude.

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CHAPTER I

LITERATURE AND FORMULATION OF PROBLEM

Introduction.— At an early stage of development, the forebrain (prosencephalon) shows thicker lateral walls which are connected dorsally and ventrally by, respectively, thin roof-plates and floor-plates. The thick lateral wall is subdivided by a shallow furrow, the hypothalamic sulcus, into a dorsal and ventral area. The hypothalamic sulcus begins at the medial end of anterior commissure and it runs backward to join the definitive sulcus limitans of mesencephalon. However, some authors believe that the hypothalamic sulcus is not a cephalic continuation of the sulcus limitans. Therefore, the possibilities exist that the hypothalamus is either a derivative of the basal lamina or a derivative of the alar lamina. The general consensus is that the hypothalamus originates mainly or entirely from a proliferation of the alar lamina.

Since the hypothalamus has correlating functions, the view that it develops from the alar rather than the basal lamina is acceptable.

The rostral part of the thick lateral walls of the prosencephalon gives rise to the cerebral hemispheres (telencephalon), while the remaining caudal part is termed the diencephalon which differentiates into the thalamus, epithalamus (pineal body and habenular complex), metathalamus (geniculate bodies), subthalamus, and hypothalamus. In advanced stages of development, the latter two regions appears to be developed from the diencephalic area ventral to hypothalamic sulcus as

well as from the floor-plate. The proliferating cells from the mantle zone give rise to the various hypothalamic nuclei as well as to the neurohypophysis or posterior lobe of the pituitary (the anterior lobe is derived from an ectodermal diverticulum from the roof of stomodeum). The optic chiasma marks the division between structures derived from telencephalon and diencephalon. Therefore, anteriorly, the hypothalamus continues imperceptibly into the parolfactory or septal region of the telencephalon. Laterally, it is bordered by the subthalamus and internal capsule. The floor is formed, from antero-posteriorly, by the preoptic recess, optic chiasma, infundibulum, tuber and mammillary bodies. Arbitrarily, the posterior border is taken as the transition area between the mammillary bodies and tegmentum of the midbrain.

Hypothalamic areas.— Le Gros Clark (1938), on anatomical grounds, subdivided the hypothalamus into 4 frontal sections by the use of 3 frontal planes. From before backwards, the sub-regions are designated as:

1. the preoptic area — lies dorsal to the chiasma and reaches forward to the lamina terminalis. Dorsally, it continues imperceptibly with the septal area.
2. the supraoptic area — above the optic chiasma and rostral to it.
3. the infundibular area — behind the chiasma and in relation to the infundibulum.
4. the mammillary area — occupies the posterior extremity of the hypothalamus.

Within the individual above-mentioned areas, a complicated conglomeration of nerve cells can be delineated. The majority of the

supposedly nuclear masses are ill-defined and diffuse and therefore are distinguished only on topographical basis when related to certain fiber tracts, while the remaining few are easily distinguished by their characteristic topography as well as by their cytological features.

Anatomical subdivision -

a) Preoptic area:
- medial preoptic nucleus
- lateral preoptic nucleus

b) Supraoptic area:
- supraoptic nucleus
- paraventricular nucleus
- suprachiasmatic (arcuate) nucleus
- anterior hypothalamic nucleus

c) Infundibular (tuberal) area:
- ventromedial hypothalamic nucleus
- dorsomedial hypothalamic nucleus
- lateral hypothalamic nucleus
- posterior hypothalamic nucleus

d) Mammillary area:
- medial mammillary nucleus
- lateral mammillary nucleus
- median mammillary nucleus
- premammillary nucleus
- supramammillary nucleus

The substantia grisea centralis hypothalami refers to the scattered cells which permeate the whole hypothalamic area.

Malone (1910, 1912, 1914), Rioch et al (1940), and Fulton (1949) and other writers have given elaborate classifications and groupings of the nuclear masses based on cell type.

Crosby's (1939) classification is based on horizontal sections and distinguishes three areas:

1. the periventricular area which is close to the ependyma of the third ventricle,
2. the medial hypothalamic area medial to the columns of the fornix.
3. the lateral hypothalamic area lateral to the columns of the fornix.

It is generally agreed that the hypothalamus contains vegetative centers (Karplus and Kreidl 1912; Bard 1928 and 1934; Cannon 1929; Ranson 1934 and 1937; Kabat, Anson, Magoun and Ranson 1935; Foerster 1936; Bronk, Lewy and Larrabee 1936; Magoun, Ranson and Hetherington 1938; Le Gros Clark 1938; Auer 1948 and 1951; and many others) which regulate the parasympathetic outflow of the brain stem and spinal cord through neuron mechanisms. It is still an open question how the humeral mechanism plays a role in this region.

The nuclear configuration of the hypothalamus of the monkey is described in the work of Grunthal (1931), Crough (1934), Papez and Aronson (1934), Le Gros Clark (1938) and Ingram (1940), and by Mettler (1948) in man.

The hypothalamic nuclei

a) The preoptic region.— Clark (1938) and Auer (1951) point out that the preoptic region is truly a part of telencephalon as it develops from telencephalon medium between the outpocketing telencephalic vesicles and diencephalon. The above authors have included this area with the main area of hypothalamus because topographically, morphologically and functionally, they are closely related. The area is relatively small and is demarcated posteriorly by the optic chiasma and anteriorly

by the lamina terminalis.

The medial preoptic nucleus continues into the adjacent septal area (paraterminal and parolfactory of human anatomy) of the frontal lobe, the nucleus of diagonal band of Broca and with the supraoptic area of the hypothalamus. The nucleus consists predominantly of small-sized cells among which are scattered medium-sized cells.

The lateral preoptic nucleus is a diffused thin sheet of gray matter composed of small and medium-sized cells also. It continues posteriorly without any structural hiatus as the ill-defined lateral hypothalamic area (nucleus) and continues caudally as far as the tegmentum of midbrain. The medial forebrain bundle, a diffused system of predominantly unmyelinated fibers, courses through this flattened layer of cells and it runs in the same antero-posterior direction.

According to Krieg (1932, in the rat), the bundle reaches as far caudally as the midbrain. It has been described in many forms and by many observers, as for example, Kappers, Huber and Crosby (1936) and Ingram (1940).

b) <u>The supraoptic middle region</u>.— This region includes the supraoptic, paraventricular, suprachiasmatic and anterior hypothalamic nucleus. The area extends approximately from a level immediately anterior to the optic chiasma to the anterior border of the infundibulum. In higher vertebrates, the supraoptic and paraventricular nuclei are most constant and very well defined. In lower vertebrates, fishes and amphibians, the two nuclei are represented by a common cell mass, the nucleus praeopticus. In lower vertebrates, the phylogenetic derivation of the nuclei from a common stem is thoroughly discussed by Meyer (1935),

Nauta and Dankmeyer (1944).

Topographically, the two nuclei differ very much as the paraventricular nucleus lies close to the ependyma of the third ventricle, while the supraoptic nucleus is superficial and close to the pia.

Strands of cells, identical with the cells of the two nuclei, may be observed which seem to connect the two nuclei. Some authors have referred to this strand of cells, which course upwards and backwards, as the accessory supraoptic nucleus. Five poorly defined subdivisions have been given to the supraoptic nucleus by Roussy and Mosinger (1934).

The position of the supraoptic nucleus is well established. The main portion lies immediately dorsal to optic chiasma and a short distance rostral to the latter while a less dense smaller portion continues downwards for a short distance in the anterior wall of the infundibular recess.

Early description of the supraoptic nucleus reaches as far back as 1872 when Meynert referred to it as the "basal optic ganglion". This basal optic ganglion was figured by Forel (1877) and first designated as the supraoptic nucleus by Lenhossek (1887), to which he included the now called lateral nuclei of the tuber (man).

Attempts to analyze the nucleus were made at first on human material by the application of the Weigert stain, while the Nissl stain was at first applied to the supraoptic nucleus by Malone (1910).

Disregarding diversity of connections and functions, on the basis of patterns of Nissl granules, Malone (1910) considered all the hypothalamic nuclear groups as being vegetative in nature. This matter

has been elaborated by Kappers, Huber and Crosby (1936).

In transverse sections, the paraventricular nucleus is seen as a column of cells which are in close relation to the column of the fornix laterally and to the wall of the third ventricle medially. In sagittal sections, the nucleus has a triangular configuration with its base directed dorsally and laterally, in close relation to the ventral medullary lamina of the thalamus and therefore in proximity to the midline nuclei of the thalamus. The dorso-lateral angle reaches into the dorsal part of the zona incerta of the subthalamus. This dorso-lateral extension is permeated by fine unmyelinated fibers which stream from the zona incerta.

The cells of this nucleus are cytologically similar to those of the supraoptic nucleus except that small cells are found among the deeply staining magnocellular portion. Due to its proximity to the periventricular nucleus, the small intermingling cells of the paraventricular nucleus may belong to this periventricular system.

Marie and Leri referred to the paraventricular nucleus as the noyau cellulaire de la substance grise centrale. Laruelle (1934) included with it the small celled suprachiasmatic nucleus. Further mention has been made by Nicolesco and Nicolesco (1929) and by Roussy and Mosinger (1935) who added to the major magnocellular portion of the nucleus certain smaller cell groups.

This author, on a functional, topographical and cytological basis, has subdivided the nucleus into three major portions (vide infra). Cytologically, the supraoptic and paraventricular nuclei are identical in many respects. Both masses contain extremely large, deeply-staining

cells. The Nissl substance is concentrated at the periphery of the perikaryon while the perinuclear area is free of precipitate (toluidine blue or cresyl violet stain). Occasionally, cells are seen which are multinucleated and which contain vacuoles. This fact led Scharrer and Gaupp(1933) and Roussy and Mosinger (1934) to conclude that these cells had secretory or excretory functions. The former authors described endocellular capillaries penetrating the magnocellular elements which suggests an unusual vascular relationship. Vacuolation and similar vascular arrangement has been reported for other hypothalamic nuclei.

It appears reasonable to criticize the above findings of Scharrer and Gaupp(1933) on the grounds that secretory activity is incompatible with the structure and functions of a neuron (Finley 1938, 1939), that the morphological indications are inconclusive and therefore a possible artefact, or possibly degenerative changes (Florentin, 1934), or post-mortem changes, and lastly, that the colloid has migrated from the hypophysis cerebri into the hypothalamic nuclei guided by the hypothalamo-hypophysial fibers which originate from the above-mentioned nuclei. However, the latest work of Bodian (1951) seems to substantiate the work of Scharrer and Gaupp.

Finley (1940) suggested as an alternative view that the cells of supraoptic and paraventricular nuclei are chemoreceptors, and Verney (1947) ascribes osmoreceptor functions to the supraoptic area.

The suprachiasmatic nucleus is a condensation of cells within the midline and dorsal to the chiasma. Topographically, it continues imperceptibly rostrally as the medial preoptic nucleus and dorsally as the periventricular nucleus (cat) while in man it is practically indistinguishable.

The anterior hypothalamic nucleus is absent in man while in mammals (cat) it is a small area of scattered cells dorso-lateral to the suprachiasmatic nucleus and the periventricular hypothalamic area closely associated with the antero-medial thalamic nucleus.

c) The infundibular area.— The ventro-medial hypothalamic nucleus is made up of medium-sized, round or oval cells and is topographically the closest to the hypophysis cerebri. It lies caudal to the suprachiasmatic and supraoptic nucleus, in direct line with the former, ventral to the dorsal hypothalamic nucleus and antero-lateral to the posterior hypothalamic area. The fornix is closely applied to its lateral surface.

The dorsomedial hypothalamic nucleus is much more diffused and smaller than the ventromedial nucleus. Anteriorly, the dorsomedial nucleus tapers and continues imperceptibly with the medial preoptic nucleus; antero-superiorly lies the periventricular nucleus, ventrally, the ventromedial hypothalamic nucleus, and posteriorly, the posterior nucleus of the hypothalamus.

The lateral hypothalamic nucleus is an extensively continuous flat sheet of small, ill-defined layer of gray continuous anteriorly with the lateral preoptic nucleus and posteriorly with gray matter of tegmentum. Therefore, it lies lateral to the plane of anterior column of fornix and medial to subthalamus. In mammals (cat), the extensive cellular area is topographically well defined by the many short, non-myelinated fibers (M.F.B.) that permeate it.

The nucleus diminishes in primates but in it new and not well understood elements, the tuberal nuclei, develop which are absent in

lower mammals and which in man produce two or three small tubercles which are easily recognized on the surface on each side between the infundibulum and the optic tracts.

The posterior hypothalamic nucleus is a diffuse small cellular area which lies posterior to the ventromedial and dorsomedial hypothalamic nucleus. The fornix and mammillo-thalamic tract border it laterally, while medially, it lies against the ependyma of the lateral wall of the third ventricle. Behind, it continues with the gray matter of the midbrain tegmentum.

d) The mammillary area.— Topographically, it is the most differentiated area of the hypothalamus. In mammals, and especially in the primates, the area is composed mainly of two hemispherical bodies and the immediate adjacent parts of posterior aspect of floor of third ventricle. The main, well circumscribed, medial nucleus consists of a homogeneous mass of small cellular elements. The nucleus is encapsulated by a distinct capsule of myelinated and unmyelinated fibers. Lateral to the main medial nucleus is a slight condensation of large scattered cells which form the lateral mammillary nucleus (nucleus intercalatus).

Carnivores shows a distinct nucleus, premammillary nucleus, which lies at the posterior end of the ventromedial hypothalamic nucleus and at the lower end of the posterior hypothalamic nucleus. The cellular elements are few but distinct in outline.

Lying dorsal to the medial mammillary nucleus is the thin triangular shaped supramammillary nucleus which seems to continue posteriorly with the interpeduncular nucleus in the posterior perforated

substance.

The median mammillary nucleus is a scattered, small mass of cells lying between the two medial nuclei. In frontal sections, it appears triangular in shape with the apex directed dorsally between the medial nuclei and the base abuts against the postero-ventral aspect of capsule of the mammillary body.

Fiber Connections of Hypothalamus.— The hypothalamus contains many fiber tracts. The great majority of these tracts are heavily myelinated and therefore have been subjected to careful considerations by research workers not only from the comparative aspect but also, to a lesser degree, by experimental methods. These large myelinated and distinct tracts make up a small area of the hypothalamus. The rest of the hypothalamus is permeated by a confusing, intricate meshwork of fine myelinated and to a greater extent unmyelinated fibers. In view of this, it is extremely difficult to work out connections of a specific area or of a specific nuclear mass.

Therefore, when efferent or afferent connections of the hypothalamus are mentioned, it should be kept in mind that the direction of conduction is arrived at solely on theoretical grounds unless the bouton method has been used. This method is absolutely reliable for it stains selectively degenerated unmyelinated and finely myelinated fibers. Reservations on the application of the retrograde chromolytic method and the disadvantages have been thoroughly discussed in Chapter II.

Therefore, the evidence for the existence of many of the connections mentioned below is unconvincing and speculative. Ingram's (1940) remark is worth quoting:

> One must appreciate that when the general pattern of this area (hypothalamus) is finally correctly and completely disclosed, there is a chance it will prove more complex than the most fanciful picture as yet presented.

With minor modifications, Ingram's (1940) classification on hypothalamic connections is adopted here. The connections have been taken up as follows:

1. Medial forebrain bundle.
2. Cortico-hypothalamic fibers.
   a) Fornix
   b) Inferior thalamic peduncle
3. Stria terminalis.
4. Supraoptic commissures (ventral hypothalamic decussations)
   a) Dorsal supraoptic decussation pars dorsalis (Ganser)
   b) Dorsal supraoptic decussation pars ventralis (Meynert)
   c) Ventral supraoptic decussation (Gudden)
5. Lenticulo- and subthalamo-hypothalamic connections.
6. Mammillary peduncle.
7. Mammillo-thalamic tract.
8. Vago-supraoptic connections.
9. Periventricular system.
10. Hypothalamo-hypophysial connections.
11. Intrahypothalamic connections.

Hypothalamic Tracts:

1. The medial forebrain bundle is a longitudinal tract which is intermingled with the cellular constituents of the lateral preoptic and lateral hypothalamic area. It is made up primarily of fine, unmyelinated fibers. It connects, according to Kappers, Huber and Crosby

(1936), the ventro-medial base of hemisphere with the preoptic hypothalamic area and the region dorsal to this area. These authors further state that the bundle carries both ascending and descending stimuli. Gurdjian (1927) assigns several components to the bundle: septo-, latero-cortico-, strio-, tuberculo-, parolfacto- and olfacto-hypothalamic fibers and the olfacto-mammillary tract which reaches the lateral mammillary nucleus. It is difficult to resolve the bundle into all its components and its most caudal termination is not clear. However, in the rat, Krieg (1932) described a component which reaches the midbrain. These caudally extending tracts are considered as separate fiber systems by Crosby and Woodburne (1951) (the anterior and posterior hypothalamotegmental tracts).

During its caudal course, in the rat, the tract diminishes progressively which led Gurdjian to believe that the medial hypothalamic nucleus receives the major contribution. Krieg (1932), working also on the rat, thought that most of the fibers of the medial forebrain bundle arise from the hypothalamic nuclei. He added a subfornical component which is specifically related to the hypothalamus. Krieg suggests a contribution to the supramammillary decussation.

Further mention of the bundle is made by Roussy and Mosinger (1934 and 1935); they include the preoptico-hypothalamic tract.

Anatomical experimental evidence does not exist which would suggest that cortico-hypothalamic fibers travel within the medial forebrain bundle. Fulton and Ingraham (1929) favour such a connection while in hemi-decorticated dogs, Papez (1938) found the bundle of normal size. In a totally decorticated cat, which had also received extensive destruction of the olfactory areas and total destruction of caudate and

lentiform nuclei, Bard and Rioch (1937) did not find the medial forebrain bundles.

It is generally agreed that the hypothalamus contains primary subcortical vegetative centers (Karplus and Kreidl 1912; Bard 1928 and 1934; Cannon 1929; Ranson 1934 and 1937; Kabat, Anson, Magoun and Ranson 1935; Foerster 1936; Bronk, Lewy and Larrabee 1936; Magoun, Ranson and Hetherington 1938; Magoun 1940; Auer 1948, and others). It has been thought that the medial forebrain bundle might carry fibers from higher centers to the hypothalamic vegetative preganglionic centers (Ranson 1937; Ranson and Magoun 1939; Magoun 1940).

2. Cortico-hypothalamic fibers.— Various observers e.g. Le Gros Clark (1948) and Von Bonin and Green (1949), have brought forth inconclusive evidence for the existence of cortico-hypothalamic fibers. Experimental findings of Bard on sham rage, and the clinical observations of Davidson and Kelman (1939) indicate that the hypothalamus, to some extent, is under the control of the neocortex.

It is known that the evidence on direct cortico-hypothalamic connections is restricted only to the fornix and certain olfactory systems. Therefore, the neo-cortical control over the hypothalamus may be exercised over fibers whose anatomical substratum has not yet been worked out. It is possible that this substratum is in the form of short neuron relays or of long, dispersed, extremely fine unmyelinated fibers.

The inferior thalamic peduncle which is made up of fine myelinated fibers skirts the rostral hypothalamic area. Houssy and Mosinger (1934) assumed that these fibers arise from the temporal lobe cortex.

Substantiating evidence has not been offered so far in the literature. It is more likely that the fibers of the inferior thalamic peduncle arise from the dorso-medial nucleus (Papez 1941) and submedial thalamic nucleus (Morrison 1930). To some extent the inferior thalamic peduncle arises also from the substantia innominata and important contributions may also come from the antero-medial thalamic nucleus (Papez 1941).

Nicolesco and Nicolesco (1929) place great emphasis on the inferior thalamic peduncle as a direct cortico-hypothalamic connection. The fronto-tuberal tract of Greving (1927) coursing with the ansa peduncularis is even more doubtful as to its site of origin.

Using induced degeneration, myelinated fronto-thalamic fibers were not observed in the macaques by Levin (1936). In the rat brain, using the Marchi stain, Milliser (1932) was unable to detect neocortico-hypothalamic fibers. Therefore, the anatomical substratum for direct neopallial-hypothalamic connections is in need of further investigation.

Excessive evidence has accumulated substantiating indirect pathways between the neocortex and the hypothalamus. These pathways have been discussed by Clark (1938) and Ranson and Magoun (1939). The connections probably course via the septal nuclei as relay stations. Indirect evidence suggests a septo-hypothalamic pathway by way of the medial forebrain bundle. Experimental evidence of Wallenberg (1934) indicates that a neo-cortico-septal tract (Marchi preparations) degenerated after frontal lesions in guinea pigs. A cortico-septal tract has also been described by Mettler (1935) on the basis of Marchi work on monkeys. Clark (1938) is inclined to believe that the cortico-

hypothalamic tract from the frontal lobe is relayed through the cellular elements of the zona incerta.

Marchi experiments by Mettler (1935), Levin (1936) and Walker (1938) have conclusively proven that fibers arise from the frontal cortex and reach the dorso-medial thalamic nucleus. Clark (1932 and 1933) has given further evidence that the above thalamic nucleus relays the frontal fibers to the hypothalamus through the periventricular fiber system. The fibers from the dorso-medial thalamic nucleus are believed to be relayed via short neurons for the periventricular fibers are unmyelinated and therefore cannot be traced by the Marchi method. With lesions in the hypothalamus, through the application of the retrograde cellular degeneration, Walker (1936) has demonstrated conclusively the existence of direct afferent pathways from the dorso-medial and midline thalamic nuclei to the hypothalamus. The source of these afferent fibers is the magnocellular part of the dorso-medial nucleus (Walker 1937).

The frontal lobe projects to the ventral and lateral nuclei of the thalamus. Conclusive evidence has not been reported on projections from these nuclei to the hypothalamus though Crouch and Thompson (1938) report degenerated fibers coursing to the hypothalamus after a lesion of the nucleus ventralis postero-lateralis. The above authors do not state the course of these fibers though some of them enter the periventricular system. Nicolesco and Nicolesco (1929) add to the observations that thalamo-hypothalamic fibers are poorly systematized. In contrast to the above authors, Greving mentions a thalamo-infundibular tract in the form of a fasciculus which connects the thalamus with the supraoptic and the tuberal nucleus and with the substantia grisea.

Laruelle (1934) does not agree with this author while Roussy and Mosinger (1934) make mention of an elaborate system of connections, i.e. the internal thalamo-hypothalamic periventricular tract which arises from the periventricular nucleus and the external thalamo-hypothalamic periventricular tract which arise from the medial thalamic nucleus; the internal and external lamello-hypothalamic tracts which arise from the intralamellar nuclei and via the thalamic fasciculus respectively. The apparent thalamic connections of the posterior hypothalamic area and the fine myelinated and unmyelinated fibers that pass from the thalamus to the region of dorso-medial hypothalamic nucleus are in accordance with these periventricular fasciculi. The inferior thalamic peduncle carries fibers from the medial thalamic nucleus (Papez 1938). This tract is closely associated with the supraoptic nucleus and Kolliker is inclined to believe that many fibers leave the supraoptic nucleus, course dorsally to enter the inferior thalamic peduncle which skids the nucleus in its dorso-lateral course. This peduncle is also closely associated with the lateral tuberal nucleus. Therefore, also the indirect cortico-hypothalamic tract requires further study.

a) Fornix.— The fornix arises in the hippocampus and is considered as a direct cortico-hypothalamic system. It was first studied by Meynert (1871 and 1872) and Gudden (1881), and later considered in detail by Kappers, Huber and Crosby (1936). The above authors maintain that it contains fibers which are said to end primarily in the medial and lateral mammillary nuclei (Edinger and Wallenberg 1902) with some fibers reaching the tuber rostral to the mammillary nuclei. Papez (1938) reports a case of a hemidecorticated dog (with 1/3 of hippocampus and fornix removed) wherein the fornix was shortened and therefore only reached the tuber, confirming thereby Edinger and Wallenberg's

observations on tuberal connections. Other workers, Roussy and Mosinger (1934, 1935) not only agree with the above authors on the tuberal component but go further and assign to the fornix specific components ending in the paraventricular and supraoptic nuclei.

Meyer and Sprouce (1951) have denied such connections on the basis of experimental approaches correlated with terminal degeneration.

b) The inferior thalamic peduncle.— This bundle is a fine myelinated fascicle coursing along the medial edge of the genu of the internal capsule. The bundle is most distinct in rostral regions of the diencephalon.

The peduncle seems to originate from the medial portion of the dorso-medial thalamic nucleus (Papez 1941) and from the nucleus submedius (Morrison 1929). Papez (1941) is also inclined to believe that important connections are present within it from the medial portion of the anterior nucleus. The bundle supposedly connects the above structures with the temporal and the insular cortex as well as with the pallidum. However, Rumbles and Papez (1948) do not mention degeneration of its bundles after temporal lobectomy in the monkey.

The fibers of the peduncle spread out in an antero-posterior direction and give rise to two limbs. The posterior limb remains in the subthalamus and extends posteriorly into the zona incerta which appears to be a synaptic area. In this zone it lies between the lenticular and thalamic fasciculus from where it fades out to the posterior end of the pallidum. The peduncle and the lenticular fasciculus together form the ansapeduncularis.

The anterior limb has a forward and lateral course under the

anterior limb of the internal capsule.

Gurdjian (1927) was not certain about the relative numbers of cortical or striatal fibers within this peduncle. On normal and experimental opossum material, Bodian (1940) suggests that many of the fibers of the inferior thalamic peduncle are of striatal or pallial origin. Gurdjian described connections with some of the midline nuclei as well as the ventro and dorso-medial nuclei. Bodian (1940) mentions the same nuclei with special emphasis on the dorso-medial and the sub-parataenialis nucleus. The latter nucleus corresponds to the medial part of the nucleus medialis ventralis of Gurdjian.

Experimental evidence is lacking on the exact origin of this peduncle. Greving (1927) mentions a fronto-tuberal component within the fasciculus to the tuberal nuclei. A cortico-supraoptic component related to the inferior thalamic peduncle is described by Nicolesco and Nicolesco (1927). Laruelle (1934) mentions the peduncle as a hypothalamic connection. According to Rousay and Mosinger (1934), the peduncle has afferent fibers from the temporal lobe to the anterior hypothalamic area and to the supraoptic nucleus. It might also contain hypothalamic fibers from the lateral hypothalamic area.

These authors mention a more ambiguous tract, the fasciculus ansa-hypothalamicus. These fibers might be the aberrant component of the ansa.

Clark (1938) suggests that the medial and lateral preoptic nuclei, which receive fibers from various tracts which arise from diverse cortical and subcortical regions, give a contribution to the inferior thalamic peduncle. This connection may be the link between the

preoptic and medial cell groups of the dorsal thalamus.

In man, Papez, Bull and Stotler (1944) report in human series J.H.C. softening which destroyed the large part of the right medial nucleus of the thalamus. These authors found the inferior thalamic peduncle completely degenerated. Both the anterior and the posterior limbs appeared devoid of myelin and fibers. They further reported that the fibers of the zona incerta were completely degenerated and that the narrow area of the incerta was occupied by broad perivascular lacunae. Papez (1943), in case J.C., and Rundles and Papez (1937) in series M.S. report of vascular disorders of the medial thalamic nucleus and the pallidum respectively. In series J.C. the inferior thalamic peduncle was partly demyelinated and reduced in size while in series M.S. the fibers between the pallidum and incerta were severely demyelinated, (Marchi stain). These cases suggest that the inferior thalamic peduncle may contribute fibers to the pallidum. In lesions involving the medial and medio-ventral nuclei, it was observed that the inferior as well as the anterior thalamic radiations undergo degenerative changes. From their close association, Papez (1941) suggests that the fibers of the inferior thalamic peduncle may be collaterals of the thalamo-cortical fibers.

3. Stria terminalis.— Ramon y Cajal (1911 - p. 479) followed the fibers of the stria terminalis to his "noyau superior" and "noyau posterior" of the hypothalamus which correspond respectively with the dorso-medial and the posterior hypothalamic nucleus. However, he did not find the terminal aborizations of these fibers and his brief note does not mention which component of the stria terminalis was involved.

As the tract passes caudally, it contributes fibers, according to Krieg (1932), to the periventricular area of the preoptic and the anterior hypothalamic region. In this region, fibers are apparently connected with the medial, and part of the lateral, paraventricular nucleus.

Laruelle (1934) has mentioned fiber connections to the lateral tuberal nuclei, the paraventricular nucleus and the substantia grisea centralis. Roussy and Mosinger list elaborate connections of the stria terminalis with practically all the hypothalamic nuclei reaching as far caudally as the medial mammillary nucleus.

Fibers arising from the stria medullaris component of the stria terminalis (component 5 of Johnston (1923), part 5x of the component as described by van der Sprenkel (1926) and to the dorso-amygdalo-hypothalamic tract of Bodian (1940)) connects, in the opossum, the reticular nucleus (van der Sprenkel) and the dorsal hypothalamic nucleus. While other fibers pass dorsal to this nucleus to enter the periventricular system to terminate in the paraventricular nucleus (Loo 1931, fig. 62, tr. ret. pv.), dorso-medial hypothalamic nucleus and in part to anterior hypothalamic nucleus (Bodian). Van der Sprenkel's (1926) detailed analysis of the stria terminalis in the opossum considered this bundle to be a system which probably arises in the reticular nucleus (Gitternucleus) and terminates in the lateral and medial parts of the tuberculum olfactorium. Other connections are mentioned; one in particular is listed as travelling with the stria medullaris to reach the supraoptic nucleus.

From the comparative point of view, the bundle has been thoroughly described by Kappers, Huber and Crosby (1936). Clark (1938) mentions hypothalamic connections of the stria terminalis to all hypothalamic nuclei including the suprachiasmatic nucleus.

According to these authors, the stria can be subdivided into various components, that is, a commissural component which interconnects the amygdaloid nuclei, and preoptic and hypothalamic components which convey fibers from the amygdala to these regions. These fibers may loop over or course behind the anterior commissure. Those fibers that do loop over the commissure are said to join the medial forebrain bundle for a caudal distribution. Kappers et al. believe that the fibers of the stria terminalis are distributed to all major hypothalamic areas as far caudally as the periventricular system and the nuclei suprachiasmaticus, paraventricularis and supraopticus.

4. Supraoptic commissures (inferior hypothalamic decussation)

a) The dorsal supraoptic commissure, pars dorsalis.— This system is frequently referred to as Ganser's commissure which is composed primarily of tortuous and extremely coarse myelinated fibers with a few fine fibers dispersed among the coarse. The system crosses caudal to the chiasma at the floor of third ventricle.

It was first recorded by Meynert (1872) and Forel (1877) and later elaborated by Ganser (1882). The latter author gave a classical description of this system in the mole and noted some of its most characteristic features which are also apparent in higher mammals and primates. Ganser recognized that the fibers of his "anterior subthala-

mic decussation" were characteristically the coarsest in the vicinity (3 micra in diameter) and that all of the fibers were not wholly separated from the more ventrally placed commissure of Meynert. Therefore, he divided the commissure into two parts, an antero-ventral and postero-dorsal. Ganser was able to follow the commissural fibers to the zona incerta and also into the interstices of the internal capsule. Ganser quoted Schnapfhagen (1877) who traced comparable fibers into the medial longitudinal fasciculus, as have other workers (see Foix and Nicolesco 1925, and Papez 1938). Ganser's description of this system was confirmed by Darkschewitsch and Prihytkow (1891) in the mole. These authors worked on a cat with a unilateral lesion of the ventral portion of the commissure (Meynert's in Gurdjian 1927 terminology) and noted the disappearance of the fibers of the dorsal part of the decussation of the opposite side. They also followed the fibers of the ventral portion to the lenticular nucleus, and the fibers of the dorsal part to the region of the fornix column in the dorsal area of hypothalamus.

The fine fibers of this fasciculus appear to arise from the lentiform fasciculus and to turn ventrally in the hypothalamus to reach the ventro-medial hypothalamic nucleus. Dejerine (1901) reports the lentiform nucleus as the site of origin, which passes medial to the fornix as the fasciculus of the tuber cinereum. Greving (1927) believes that the fibers arise from the lenticular fasciculus and assigns to them no hypothalamic connections. Gurdjian (1927) considered the commissure as arising from the subthalamic and the nucleus supraopticus diffusus. Krieg (1932) thought that the fasciculus is closely related to the reticular nucleus of the thalamus and the zona incerta,

while Roussy and Mosinger (1934) give the fasciculus a more wide distribution which includes the anterior hypothalamic area, the nucleus supraopticus diffuses, the dorso-medial hypothalamic area and with specific connections between the nucleus of Darkschewitsch and the tuberal nuclei. Vonderahe (1937), in certain human cases, described an anomalous commissure bridging the third ventricle between the areas containing the paraventricular nuclei. The author suggests that this evidence points to a probable termination, presumably of the dorsal supraoptic commissure, in the paraventricular nucleus in normal brains. Clark (1938) relates the dorsal supraoptic commissure to the anterior and lateral hypothalamic nuclei and to the ventro-medial hypothalamic nucleus with fibers extending to the subthalamus. Further elucidation is given by Kappers, Huber and Crosby (1936) who describe in this system short commissural fibers which interconnect opposite hypothalamic regions together with fibers of the tegmentum and the medial longitudinal fasciculus.

b) Dorsal supraoptic decussation pars ventralis.— In general, it represents the so-called commissure of Meynert. The bulk of the supraoptic decussation consists of several diffused groups of fibers of varying calibers and with diverse terminations. Weaver (1937) in a study on the cat described the decussations as a unit and referred to it as the inferior hypothalamic decussation.

According to this author, these fibers, in and near the midline, form a unitary system of fibers, of varying sizes, which cross in the posterior part of the optic chiasma and behind the chiasma. They remain medial to the optic tract and enter the medial part of the cerebral peduncles, interweaving as small bundles between the medial

elements of the cerebral peduncle. The further course of Meynert's commissural fibers is not clear. Ramon y Cajal (1911, Fig. 255) described collaterals of certain optic fibers, which he considered to be part of Gudden's commissure, arborizing within his "noyaux de la bandelette optique". This nucleus corresponds to the ento-peduncular nucleus of the opossum, Bodian (1940). Papez (1939) is inclined to believe that the commissure is a primitive optic connection relating the geniculate bodies to the supraoptic nucleus and other hypothalamic structures of the opposite side.

Kappers, Huber and Crosby (1936) mention no hypothalamic or geniculate connections for higher mammals but mention crossed tecto-subthalamic and lenticulo-subthalamic components.

Nicolesco and Nicolesco (1929) describe connections with the lateral hypothalamic area and supraoptic nucleus. Clark's (1938) experimental evidence indicates connections with the globus pallidus and contributions to the medial forebrain bundle. Weaver (1937) mentions no pallidal origin but suggests subthalamic, pretectal and geniculate (medial) relationships. Hypothalamic connections are not mentioned. Laruelle denied that any of the supraoptic commissures were of importance to the hypothalamus. In spite of the close relationship of the fibers to the supraoptic nucleus, no evidence for endings is found by Ingram (1940).

c) <u>The ventral supraoptic commissure of Gudden</u>.— It lies among the fibers of the optic chiasma and Weaver (1937) could not find such fibers in cats with optic fiber degeneration after enucleation of the eyes. Moeli (1904), Marie and Leri (1905) and Herzog (1906) found

no trace of it in human specimens with complete optic degeneration.

5. Lenticulo and subthalamo-hypothalamic connections.--

Kappers, Huber and Crosby mention the lenticular fasciculus and the inferior thalamic peduncle as the possible stratum of connection. Papez (1937, 1938) has a connection through Ganser's commissure from the globus pallidus to the ventro-medial hypothalamic nucleus. Spiegel (1932) gives no anatomical substratum but states that the chief afferent hypothalamic connection is with the corpus striatum. Gurdjian (1937) and Krieg (1932) give the medial forebrain bundle (in the rat) as the possible anatomical connection between the two elements. In man, Laruelle (1934) mentions striato-hypothalamic fibers through the ansa peduncularis and the ansa lenticularis. Greving (1927) implies striatal connections through the doubtful strio-peduncular tract. Probst and Monakow, as cited by Wilson (1914) offer the lenticular fasciculus as a connection to the tuberal nuclei. Pallidal lesions in cats led Morgan (1927) to a description of degenerated fibers (Marchi) passing through the medial third of the cerebral peduncles to turn medially at Forel's field H2 (lenticular fasciculus). This fasciculus corresponds to Krieg's hypothalamic fasciculus (rat), and pallido-hypothalamic fasciculus of Bard and Rioch (carnivora). If the fibers actually end in the hypothalamus, they appear to enter the ventro-medial hypothalamic nucleus as seen after destruction of the globus pallidus. This experiment of Bard and Rioch also demonstrates the absence of crossing lenticular fibers within the supramammillary decussation (which may be concerned with decussating fornical fibers?). Woollard (1932) cites a lesion of the subthalamic nucleus in a cat which resulted in the degeneration of

fibers in the opposite hypothalamic areas.

6. Mammillary peduncle.— Edinger and Wallenberg (1902) observed ascending degenerating fibers within the peduncle after placing a well circumscribed lesion within the nucleus gracilis. Kappers (1921) thought that it represents the spino- and bulbo-hypothalamic tracts of lower vertebrates which topographically are closely associated with the lemniscal system in mammals. Wallenberg (1900), Probst (1902) and Papez (1923) have brought forth experimental evidence to show the ascending component within the peduncle. Clark (1938) believes that certain descending fibers may be also present, and Tello's study (1934) in the mouse, by the developmental approach, shows clearly that the tract emerges from the region of the lemniscal system. Probst (1901), Allen (1923), Ranson and Ingram (1932), Ferraro and Barrera (1936), Clark (1936) and Walker (1938, 1939) have been unable to support the evidence on the close relationship between the lemniscal system and the peduncle.

Gurdjian (1927) in normal rat material described the peduncle as arising from the medial lemniscus. Roussy and Mosinger (1934, 1935) include fibers from the gracile and cuneate nuclei in the peduncle to be distributed to the medial mammillary and to the supraoptic nucleus. Clark (1938) includes the mammillary peduncle as one of the main pathways for sensory impulses to reach from the lower brainstem to the mammillary body. This has not been proven by other data.

Papez (1923) lists it as one of the main afferent pathways of the hypothalamus, mainly to be distributed to the lateral mammillary nucleus and with some fibers continuing rostrally into the lateral part of the tuber (rat, Marchi studies).

Papez (1932) placed the origin of the bundle in the ventral tegmental nucleus of Tsai (1925) in the opossum. The latter author followed this tract from the lateral part of the mammillary body to a level just caudal to the oculomotor roots where the fibers appear to intermingle with lemniscal fibers. Supporting these conclusions is the work of van Valkenburg (1912) and Fortuyn (1912). They believe that in the rabbit embryo the tracts are distinct. After lesions which interrupted the mammillo-tegmental tract in the rabbit, van Valkenburg observed retrograde cellular degeneration within the dorsal part of the mammillary nucleus. Ibanez (1935) reported, on the basis of Marchi studies, that the common bundle of origin of the mammillo-thalamic and mammillo-tegmental tracts arises in the medial mammillary nucleus, as observed by most workers, but that a small mammillo-tegmental component also arises in the lateral nucleus and is independent of the bundle of Vicq d'Azyr.

Bodian (1940) reports on a direct lesion of one bundle of Vicq d'Azyr in the opossum. The results gave no evidence of fibers passing ventrally in this bundle as was described by Clark and Boggon (1923) in the rat after lesions of the antero-ventral nucleus of the thalamus.

Of interest are Tello's (1934) ontogenetic observations on colloidal silver preparations wherein the mammillo-tegmental tract appears earlier than the mammillo-thalamic tract. Similar observations have been presented by Shaner (1932).

The tract arises from the medial mammillary nucleus and in part from the lateral mammillary nucleus (Clark 1938). On the basis of Weigert material, Rioch suggests that a small component of the bundle arises from the nucleus intercalatus (his lateral mammillary nucleus).

The tract courses dorsally to the anterior nuclear complex of thalamus with the antero-ventral nucleus receiving the greatest component (primates). This nucleus has widespread afferent connections with the cingulate gyrus (neo-pallial cortex) as shown by various authors in diverse experiments, also with primates (Clark and Boggan 1935).

7. The mammillo-thalamic tract (bundle of Vicq d'Azyr).— In most mammals, the mammillo-thalamic tract is a well circumscribed bundle of myelinated fibers which course through vertically downward and ventrally within the medial hypothalamic area. It interconnects the mammillary complex with the anterior nuclear complex of the thalamus.

Phylogenetically, Clark (1938) considers the tract as a local differentiation and aggregation of the periventricular system of fibers.

Cajal (1911) described the tract (Golgi preparations in the mouse) as being collaterals of the mammillo-tegmental tract.

Ontogenetically, Tello (1934) reports (in mouse embryos stained with colloidal silver) that the mammillo-tegmental tract is evident earlier than the mammillo-thalamic tract. Shener (1932) in pig embryos reports similar observations and has further remarked on the lateness of the tract in development. Van Valkenburg (1912) showed degeneration in the mammillo-tegmental tract without any effect on the mammillo-thalamic tract.

On Weigert preparations, Rioch reports (carnivora) that the tract arises primarily from the medial mammillary nucleus and in part from the nucleus intercalatus (his lateral mammillary nucleus). Normal and experimental material shows that the tract terminates in relation to all

the nuclei of the anterior group of the thalamus (macrosmatic mammals: antero-medial, antero-ventral and antero-dorsal nuclei) of same and opposite side (Clark 1929, 1932). In higher mammals and primates, the antero-medial nucleus regresses while the antero-ventral nucleus becomes larger and more sharply circumscribed, and the mammillo-thalamic tract ends mainly in the antero-ventral nucleus which projects to the gyrus cinguli (neopallial cortex).

Experimental evidence is at hand, according to Clark (1938), that thalamo-mammillary fibers course in the bundle of Vicq d'Azyr.

8. Vago-supraoptic connections.— On the basis of the relative position of the mammillary peduncle and the secondary gustatory and visceral paths to hypothalamus in fishes, Clark (1938) has suggested that the mammillary peduncles may conduct impulses from the nuclei of IX and X nerves to the hypothalamus.

Huang (1938) demonstrated physiologically a connection between the vagus nucleus and the supraoptic nucleus. Though this pathway was not shown, Ingram suggests that it may course through the lateral hypothalamic area or even more laterally. Allen (1923) mentions the medial lemniscus as the possible pathway though this system is crossed and Huang's physiological path is uncrossed.

Further physiological evidence of a pathway between the vagal nuclei and the supraoptic nucleus is suggested by the work of Bronk, Lewy and Larrabee (1936).

9. Periventricular system.— In the cat, the periventricular fibers of the diencephalon can be sub-divided into an anterior group

and a posterior group. The latter extends caudally in the stem as the dorsal longitudinal fasciculus of Schutz.

The periventricular fibers are mostly unmyelinated and therefore cannot be detected by the Marchi degeneration method. The system lies close to the ependymal lining of the third ventricle or on either side of midline with most of the fibers having a vertical course. To reach the mesencephalic central gray, the fibers course upwards subependimally. The fibers become closely related to the medially placed nuclei of the thalamus, especially the dorso-medial nucleus (Rioch 1931).

In a series of normal mammals, including human brains, Marburg (1931) made a thorough study of the posterior periventricular system (fasciculus periependymalis). In his comprehensive account he has reviewed most of the pertinent literature on this interesting and somewhat neglected system. He emphasizes the relation of this system to the tuberomammillary and periventricular hypothalamus, to the dorsal tegmental nucleus of Gudden and to the neighbouring mesencephalic gray associated with olfactory dependencies, including the interpeduncular nucleus and several gray regions surrounding the gustatory, vagal and trigeminal centers. Krieg (1932) states in his studies on the rat that most of the periventricular fibers arise from the suprachiasmatic nucleus, the posterior hypothalamic nucleus, the ventro-medial and dorsomedial hypothalamic nuclei. Schutz (1891) adds fibers from the supraoptic nucleus. Krieg also adds an anterior component which is said to arise from the preoptic periventricular nuclei which course dorsally, close

to the ventricle to form a fascicle in the dorsal part of the massa intermedia. Beneath the posterior commissure it joins the main posterior component which arises from the hypothalamic nuclei (see above). These fibers receive contributions from the midline nuclei of the hypothalamus and some from the medial forebrain bundle. After the two components have mingled, the bundle separates, with divisions coursing dorsally and ventrally to the aqueduct. The dorsal or tectal division enters the colliculi. The ventral forms the dorsal longitudinal fasciculus which was traced as far caudally as the superior vestibular nucleus. Krieg suggests that it ends in the dorsal motor nucleus of the vagus. Little is mentioned of the tegmental nuclei. The prevailing opinion is that the fasciculus of Schutz connects with the motor nuclei of the brain stem and particularly with the vegetative motor nuclei. The posterior hypothalamic component of Krieg receives additional fibers from the supraoptic nucleus (Schutz 1891) and from the paraventricular nucleus (Loo 1931). Therefore it appears that the whole antero-posterior extent of the hypothalamus contributes in some degree to the periventricular system.

Clark's (1938) and Larusslle's description is essentially the same, except that the former author places special emphasis on the contribution from the posterior hypothalamic area which is said to be the primary source of fibers to this system (cf. Gurdjian, 1927). Nicolesco and Nicolesco (1929) refer to the periventricular system as the faisceau du tuber cinereum and ascribe its origin to the paraventricular nucleus and the ventral part of the tuber. Greving's (1927) tractus substantiae grisae infundibuli and the tractus hypothalami appear to conform to the

periventricular system and dorsal longitudinal fasciculus. Roussy and Mosinger (1934) include the dorsal longitudinal fasciculus in their external periventricular system, and Dejerine's faisceau du tuber cinereum corresponds through part of its course with the dorsal longitudinal fasciculus giving as its origin the lentiform nucleus. Kappers, Huber and Crosby (1936) ascribe the origin of the periventricular fibers to the dorso-medial and ventro-medial hypothalamic nuclei, the posterior hypothalamic area and the periventricular gray to discharge caudally to the dorsal tegmental nucleus to be relayed to preganglionic centers and other efferent centers of the stem. Additional fibers reach the tectum and dorsal thalamus. Papez and Freeman (1930) observed degenerating fibers within the periventricular system in the region of the hypothalamus after a lesion of the habenular area.

Beattie, Brow and Long's (1930) degenerating experiments on cats with hypothalamic lesions, wherein the Marchi and the Alzheimer-Mann methods were used to detect the degeneration, indicate that most of the periventricular fibers are unmyelinated. A word of caution should be added, that is, the method of Alzheimer-Mann (eosin and methylene blue) which is designated to stain degenerating axons is not very reliable.

It is apparent from the literature mentioned above that all the studies on the periventricular system have been done mostly on normal material and whenever experimental lesions were used, the Marchi method was employed to detect the degenerative changes. However, it appears (see Chapter III) that the periventricular fibers are primarily unmyelinated. Therefore, results obtained through Marchi studies should be accepted critically. This would explain the reason why the authors

mentioned above do not assign to the periventricular system a septal component. It also seems possible that the periventricular fibers may carry cortico-hypothalamic as well as cortico-septo-hypothalamic components to various hypothalamic gray masses.

As already stated, the anterior periventricular system, in general, consists of fibers which course vertically along the wall of the third ventricle, or on either side of the midline. It is best seen in horizontal and oblique sagittal sections. It connects chiefly the dorsal thalamus and perhaps the epithalamus with the periventricular gray of the hypothalamus.

In the dorsal thalamus the fascicles of the periventricular system are fine and unmyelinated. These fine unmyelinated fibers appear to be long neurons as compared to the fibers within the ventral hypothalamic region. At the latter region, the fibers are many and short. A crossing is clearly seen (cat) at the ventral angle of the third ventricle as reported by some authors.

The posterior periventricular system (fasciculus periependymalis).— The components are extensive; some fibers are finely myelinated and mostly unmyelinated. A large part descends in the central gray of the stem to form, in part, the dorsal longitudinal fasciculus of Schutz. This system corresponds to the fasciculus periependymalis of Ramon y Cajal and of Marburg, the fasciculus longitudinalis griseus of Kolliker (1896, Fig. 637), and the hypothalamic division of the periventricular system of fibers of Gurdjian (1927), Krieg (1932) and others.

It should be mentioned that Ramon y Cajal (1911) separated his fasciculus periependymalis (voie longitudinale peri-ependymaire p. 193)

into dorsal and ventral components. Of interest also is the fact, as mentioned by Ramon y Cajal, that long and short fibers and collaterals were present in the periventricular system.

10. Hypothalamic-hypophyseal connections.— In sagittal and horizontal sections, a stream of fine unmyelinated fibers is seen coursing through the infundibulum and infundibular stalk. The fibers pass caudally, medially and downwards.

Cajal (1894) first demonstrated these fibers in the mice and was able to follow them from the hypothalamus to the neurohypophysis. Retrograde chromolytic changes were observed by Lewy (1924) in supraoptic nuclei after injury to the neurohypophysis (this disproved Cajal's concept that the hypophyseal fibers conducted centripetally). Pines (1925), Greving (1925, 1927) and Stengel (1926) substantiated Lewy's work on the origin of these fibers principally from the supraoptic nucleus and, to a lesser extent, from the paraventricular nucleus. The connections were further confirmed by Nicolesco and Nicolesco (1929), Cushing (1930), Fisher et al. (1935) and Ingram (1940). The number of fibers have been estimated from 10,000 in the rat to 60,000 in monkeys, and up to 100,000 in man (Rasmussen, 1938, 1940; Magoun and Ranson, 1939; Harris, 1951).

Ingram and Fisher (1936) concluded that only few fibers cross to opposite side but Laruelle (1934) and Roussy and Mosinger (1934, 1935) claimed that a large proportion of fibers take place in the crossing.

The greatest distribution of these fibers is to the neurohypophysis with few entering the pars intermedia and pars anterior of anterior lobe (Pines 1925; Hair 1938; Brooks 1938; Harris 1948). The fibers were traced by Brooks and Aersh (1941) to specific cells of posterior

lobe and Beattie traced the fibers to their end stations around the modified glia cells, the pituicytes.

11. Intrahypothalamic connections.— Experimentally, the anatomical substratum for the interconnections among the constituent hypothalamic cell masses has not yet been advanced. Various authors have mentioned various tracts. We have found no basis for such conclusions after carefully examining many series of cats which had been stained with colloidal silver.

In normal cat material, stained with colloidal silver, fine unmyelinated fibers are seen interconnecting the median and medial mammillary nuclei but not with the lateral mammillary nuclei as suggested by Ingram (1940).

The tractus paraventricularis cinereus of Greving (hypothalamic-filiformis tract of Krieg) may be a link between the supraoptic and paraventricular nucleus. This internuclear connection might be affected through the dentrites of paraventricular cells (Nicolesco and Nicolesco 1929) or through the axons of supraoptic cells (Nicolesco 1934). These fibers might well belong to the paraventriculo-hypophyseal tract.

Roussy and Mosinger (1935) described widespread associational connections of the paraventricular nucleus which have not been substantiated. In normal rat material, Krieg (1932) described the tractus filiformis lateralis which supposedly interconnects the paraventricular nucleus with the thalamic reticular nucleus. Indications are present for this bundle in the cat as reported by Ingram, Hannett and Ranson (1932). Clark (1938) mentions connections with the habenula and dorsal

thalamus by way of stria medullaris. The supraoptico-thalamic tract of Greving (1925), which corresponds to the faisceau residuaire de la bandelette of Moeli (1904), Marie and Leri (1905) and Herzog (1906) has been traced to supraoptic nucleus from a region in front of lateral geniculate body, where its fibers diffuse towards the lentiform nucleus. These fibers might well be aberrants of the ansa peduncularis and not of optic origin as it was formerly thought by Greving. Interconnections between hypothalamic nuclei might well be accomplished through the medial forebrain bundle and/or the periventricular system which has been discussed.

Supraoptico-tuberal connections (man) have been described by Greving (1925) which are now regarded as fibers of the supraoptic-hypophyseal tract.

Interconnections among the various hypothalamic nuclei are mentioned by Auer (1952) who showed morphological evidence in the cat for the existence of short neuron relays in the hypothalamus.

The data mentioned in the foregoing account on the nuclei and fiber connections of the hypothalamus, supplemented by the data on the neuro-histological techniques (Chapter II), suggest the formulation of the following problem which is of primary importance.

1. The Nissl and Marchi methods (the most widely used for hypothalamic studies) are most useful for many purposes, though not absolutely applicable to hypothalamic studies for the following reasons:
   a) <u>The Nissl method</u>: The classical description of the Nissl granules at various stages of disintegration proves difficult in interpretation especially when one considers the fact that the

magnocellular nuclei and the posterior hypothalamic nucleus have an eccentric peripheral disposition of the tigroid substance.

b) The Marchi method: Its usefulness lies only in the studies of degenerating myelinated tracts and is therefore useless for the unmyelinated fibers of the hypothalamus.

2. Local neuro-physiological stimulation (with the aid of the Horsley-Clarke sterotaxic instrument) or the application of strychnine, acetylcholine, or other chemicals, are difficult to assess in the crowded hypothalamic area with its dense conglomeration of nuclei, tracts and short neurons. Therefore, the physiological connections are speculative and not always convincing.

3. Pathological specimens are rarely of value since the lesions are usually diffuse and undiscriminating in their involvement of fibers and nuclei.

4. Therefore, a reliable method for staining and identifying degenerated unmyelinated or finely myelinated fibers is the first prerequisite to any further work on the hypothalamus.

5. A re-evaluation of hypothalamic connections, based on strict morphological data, especially for the individual nuclear masses (the magnocellular nuclei), is necessary for an interpretation of their possible functions.

CHAPTER II

METHOD

Introduction.— As described in Chapter I, the hypothalamic region is permeated primarily by fine, short and non-myelinated fibers. Though many techniques have been devised to detect these neurofibrils, basically through the application of colloidal silver, none of them seem to fulfil this purpose ideally.

The neurophysiologists have made a great advance in studying the function of the hypothalamus. The neuroanatomists are not yet prepared to offer an anatomical substratum for the results of the neurophysiologists experiments due to the fact that the present day techniques are not applicable to degenerating non-myelinated neurofibrils and terminals.

The review of the literature shows the lack of information that prevails today in the afferent and efferent connections of the hypothalamus and on the connections of specific nuclear masses, that is the magnocellular nuclei.

Therefore, during the course of our preliminary investigations on the magnocellular nuclei of the hypothalamus, it became imperative that a reliable neurofibrillar stain had to be employed. Since systematic experimental destruction was contemplated, it implied that the degenerated processes of the neurons should also be detectable by the stain. Therefore, the neuro-histological method should demonstrate consistently the particular features of the tissue, whether the tissue be normal or in progressive stages of degeneration.

The multitude of existing methods for staining nervous elements with colloidal silver is an indication of the relative unsatisfactory nature of these techniques. The staining should work equally well with standard fixatives; always demonstrate all nervous elements and the chemicals should be simple to prepare. Most of all, the technique should work well with serial paraffin sections in order to avoid staining intensity irregularities and possible lack of cohesion between the various parts as seen in frozen sections. Thereby the advantages of thin serial sections could be maintained without tears as well as the ability to stain alternate sections with various other stains.

History of Silver Methods.— Golgi (1879) first introduced colloidal silver in the studies of nervous tissue, but it was his contemporary, Ramon y Cajal who exploited Golgi's technique to the utmost. It has been said by some authors that Cajal's methods were originally modifications of the photographic process of Simarro (1900). Today's techniques are fundamentally based on the earlier silver methods of Cajal (1903-1933) or Bielchowsky (1901) techniques. Both methods are based on the silver reducing power of certain photographic reagents, and therefore, the presence of a certain similarity of conception between the photographic process of Simarro and the neuro-histological methods of Cajal. Modifications of the formulae of Cajal and Bielchowsky have been introduced by Da Fano (1908), Kato (1908), Pusateri (1908), Besta (1910), Ascoli (1911), Huber and Guild (1913), Ranson (1914), Nonidez (1939), and Minckler (1940).

The Bielschowsky's (1902) method is well conceived from the process of "silvering" glass. If ammonia is poured into a solution of

silver nitrate, a precipitate is formed which is re-dissolved by the addition of excess ammonia. If a basic solution of formaldehyde is slowly added to this easily reducible diammoniacal-silver-nitrate ($N(NH_4)Ag\ H_2NO_3$), metallic silver is readily precipitated. Through successive modifications, Bielschowsky arrived at three modifications: for frozen sections (1904), for peripheral nerve fibers (1904) and for bulk pieces (1905).

Cajal and Bielschowsky's method and their modifications, though excellent for many purposes, all depend on block impregnation, the use of frozen sections, and a special fixative. Furthermore, all these techniques are time consuming and irregular. The worker risks all and has little control over the impregnation. Patchy staining and burning is always present, especially at the periphery of the sections.

Recently, a series of new techniques were evolved for staining serial paraffin sections. Few of these are easily performed and if well executed the results are extremely valuable, notably, the Bodian (1936) method which has made available a neurological stain of unusual merit. Freedom from precipitate and a high degree of differentiation of nerve fibers characterize sections made by this method.

The method is developed for staining mounted paraffin sections which give uniform, sharp and specific staining of nervous elements, including myelinated and unmyelinated fibers, neurofibrils and terminals (free terminals and bulbs). Unfortunately, the technique uses protargol, a strong silver proteinate, now only manufactured by the Winthrop Chemical Company. Nothing is known of its chemical composition except that it is a combination of silver with partially hydrolized egg albumen. Furthermore, only certain samples (silver albumose)

produce the desired results while others (Holmes, 1943) and even those manufactured by the same company (Green, 1947) are inactive. Holmes (1943) suggests that the Bodian method owes its success to the fact that the solution of protargol (if it is the active protargol) with added copper contains a very low concentration of silver ions and has a pH which, during the course of the stain, slowly falls from about 8.2 to below neutrality.

Silver (1942) describes a rapid silver-on-the-slide method for staining nervous tissues. The method is a modification of the standard techniques for silvering glass in mirror production. It uses a mixture of Rochelle salt, colloidal silver nitrate and potassium sulphide added to ½% protargol. The solutions are difficult to prepare; the silver is of unknown concentration and it is capricious in action.

Holmes (1943) in his extensive and thorough review of silver techniques for staining paraffin sections states that silver oxide and silver carbonate are more effective than silver nitrate. Auer and this author (1953) have done extensive studies on the above compounds and find them not as efficient as the silver nitrate. The least differentiation has been found with silver carbonate. Connective tissue elements and capillaries were always impregnated. Holmes has not tried silver chloride (a constituent of protargol) and the authors have found it totally unsuccessful. His silver nitrate solution is extremely weak (1:10,000—1:1000,000) and furthermore it employs a borax-boric acid buffer. The method has been a failure in our hands. This failure may be due to the fact that a variation in the pH and more likely in the silver concentration plays an important part.

Pearson and O'Neill's (1946) method resorts to physical development. The sections are first impregnated with colloidal silver nitrate and the deposited colloidal silver is intensified by another impregnation using silver nitrate and a mild reducing substance (hydroquinone) with a protective colloid (gelatin) added in order to arrest excess deposition of colloidal silver.

The results with this method are such that the neurofibrils are unnecessarily thicker (due to excess deposition of silver around them) and it does not appear to be highly specific as it works best with embryonic tissue.

Pearson (1947) has stated that the use of protargol with colloidal silver nitrate as a preliminary step improves the differentiation. Furthermore, gelatin is a notoriously variable substance and results may depend on the type used. The presence or absence of mustard oil or sodium chloride perhaps plays an important part towards the final differential staining.

With the Romanes method (1946) we have been able to obtain reasonably good results but not as good as with the Bodian method. Romanes liberates colloidal silver from the action of tannic acid on a weak (.1%) ammoniacal silver nitrate solution. Pyridine is used as the alkaline base. The method seems to work best for the peripheral rather than the central nervous system for which purpose it was developed.

Two variables in this method are difficult to control. As with all the ammoniacal silver nitrate solutions, it is difficult to determine the exact end point when all the silver hydroxide, precipitated by the ammonia, has just been re-dissolved. Furthermore, the type of gelatin

used and the pH of the final solution are factors which are unpredictable and therefore exert a profound effect on the differential impregnation.

Observations on Colloidal Silver Staining.— Of all the silver salts tried, the nitrate constantly gave the best results. Salt solutions of silver nitrate at various concentrations were tried. The concentration ranged from .001--10.00% of silver nitrate. With all other factors concerned, the results were best with a concentration ranging between 1.5 to 2.5%.

With less than 2% the sections appeared light brown in color; the differentiation was not as good and there was no excess deposition of silver on the sections. With a concentration of 3% to 10%, the sections are of a deeper brown color, and upon examination, it reveals granular deposits and therefore a loss of differentiation. With other trials, it was concluded on the 2% as the most appropriate concentration for differentiation of neurofibrils. This is in accordance with the routine technique (Auer-Di Virgilio, 1952 and 1953) now in use for all neurological studies.

Double impregnations with solutions containing less than 2% colloidal silver were unsuccessful. Time and temperature variations were also tested. The process failed to produce any marked increase in the intensity of the staining. It further suggested that the silver receptors, presumably reducing agents in the tissue, had been saturated with the first impregnation. This is in marked contrast to Silver's (1946) observations which led him to suggest that negatively charged silver atoms were deposited on positively charged proteins (an amphoteric

substance) and that the process was a continuous one and could be carried out till large quantities of colloidal silver had been deposited. The question as to how the neurofibrils are able to absorb more of the colloidal silver above its normal amount, and at the same time give an appropriate differentiation of the fibrils, is not clear. The findings of Holmes (1943) substantiate Silver's hypothesis but it is apparent that perhaps a totally different dynamic system is present with respect to deposition, tissue reduction and differentiation (vide infra).

This evidence points to the fact that the specifically concerned radical or radicals are the principal agents in the reduction of colloidal silver. The slow reducing radicals which are present within the nerve tissue and which participate in the reaction (chemical or physical) are the sulphidryl groups (S-S linkage), ascorbic acid and aldehydes. From the experiments of Hsu-Mu Liang (1947), who has stained fresh and fixed nerve fibers with Schiff's reagent, it appears that the aldehydes are in part responsible for the reduction phenomena.

To colloidal silver nitrate, we have added small quantities of copper. The time and temperature factors were varied and controlled. The tissue was paler and upon further treatment the neurofibrils appeared purple rather than black. The sections resembled those which had been impregnated with a small amount of colloidal silver (.001%). This suggested that the presence of copper in the colloidal silver solution removes much silver from the solution and in no way does it increase the specificity of the reaction. Nevertheless, in photographic emulsions small amounts of copper are added to increase the sensitivity to light by acting as reduction centers.

To demonstrate terminal boutons within the central nervous system, Cajal used chloralhydrate and DeCastro (1925-26) used chlocalhydrate with urethane in the impregnating fluids. Both substances seem to impair differentiation. Green (1948) added urethane to protargol and reports improvements in the staining of nerve fibers in the cerebral cortex.

The Influence of Fixatives.— The problem of fixation before treating the tissue with colloidal silver has recently been thoroughly reviewed by Bodian (1937), Davenport and Kline (1938). We have been able to attain good results with a range of fixatives. The introduction of pure unmixed acetone and modified Zenker acetic as a fixative for the central and peripheral nerve system to stain neurofibrils has been used successfully with excellent results for the first time. Other well known fixatives have been modified and advantageously used also.

Five to 10% formaldehyde has given good results. With 20% formaldehyde, it is observed that the perikaryon has a tendency to shrink which is accompanied by neuroplasmic changes which are not normally observed in the cell body. The neurofibrils appear normal but on careful comparison with preparations which had been fixed either in acetone or modified Zenker acetic, it becomes apparent that degrees of swelling had taken place.

Good results have been observed at the region of the cerebellum, the cortex, the interbrain and the stem, by adding to 10% formaldehyde, 50% alcohol. The proportions were given to us by Dr. Olszewski of the Montreal Neurological Institute where this fixative is normally used for all routine staining: Cajal, Nissl and Heidenhein.

Ten percent formaldehyde, 50% alcohol, 5% glacial acetic worked equally well. This fixative will clearly demonstrate the cell body and the nucleus.

The picro-formol-acetic (Bouin, 1897) is a strong fixative and penetration is excellent. The tissue is equally fixed, and all the delicate cytological constituents of the cell as well as fibrils are preserved adequately. Acetone-formol, ratio of 8 to 2, was used with success. The finer details of the neurofibrils are satisfactorily preserved and no detectable swelling of the perikaryon is observed but it was noticed that when the tissue was toned (1% AuCl + 3 drops of glacial acetic acid), the background of the section became a light pink color which seems to obstruct the contrasting black fibrils. In the pure 100% acetone fixed tissue, the background upon which the neurofibrils appear to rest is completely colorless to ordinary light. It is possible that the action of formaldehyde on the tissue is responsible for the pinkish color of the background.

Modified Carnoy (1886, 1887), wherein 5% glacial acetic was added to 100 cc. of alcohol, was found to be an excellent fixative for the study of neuron morphology but not as good for neurofibrils. The acetic acid did not injure the finer elements of the neuroplasm but rather brought out the nuclear structure with no signs of swelling.

The chromate salts, as well as mixtures of chromates, have been and are being studied extensively. In a future paper, the chromates used as fixatives on nerve tissue as a preparation to colloidal silver impregnation on paraffin sections will be reported. Of the salts and mixtures of

the salts with various other reagents, we have tried Muller's, Kollmann's (1885), Erlicki's (1887), Kultschitsky's (1887) ammonium chromate, calcium dichromate, chromates and alcohols, chromates and acetone, Zenker's and others.

It may now be stated that the most successful of the fixatives mentioned above for nerve tissues as a preparation for colloidal silver impregnation is modified Zenker acetic. This has given excellent results. The nerve tissue which has been fixed in the modified Zenker acetic solution was serially sectioned and mounted on slides in the usual way and was followed by the fibrillar stain of Auer-Di Virgilio (1952, 1953). The results are constant and uniform for all the regions of the central nervous system (cortex, interbrain, stem and cord). Furthermore, the solution is highly recommended for the preservation of the delicate and minute structural details of the nervous system. Terminal degenerative processes of the bulb, ring, or free ending type is detected with ease. Degenerative changes within the perikaryon can be detected even when changes are minute. Furthermore, if alternate sections are stained for the Nissl granules, its superiority as a fixative to all others, extensively tried in the laboratory, is apparent. After three years the sections have not faded at all in contrast to other fixatives in which the Nissl (Toluidine blue) stain was used.

The total shrinkage of the whole cat's brain is relatively small, 10 to 15%, as compared to the data of Hanson et al of 33%. The modified Zenker fixative leaves no residual precipitate of the sublimate on the sections and therefore no need to treat the sections with an alcoholic solution of iodine.

Ordinarily, Zenker fixed tissue is treated with 10 to 20% iodine solution for various times depending on the tissue used. Subsequently, the iodine is removed from the tissue by passing the sections through three or four changes of 80% alcohol till all the iodine is removed.

We have observed that in the iodine treated sections, the reagent acted on the tissue in a way that colloidal silver impregnation with good differentiation becomes impossible. This impediment to colloidal silver impregnation might be due to the oxidizing power of the iodine which might subsequently alter the constituent elements of the neurofibrils. We have tried various reagents to restore the tissues to their normalcy after treatment with iodine; however, the results of these have been negative.

In contrast to Zenker, acetone (100%) as a fixative causes a tremendous amount of shrinkage as it is also a powerful dehydrating agent. This fact is true for the whole brain (cats primarily). The total shrinkage is no longer observed after twenty-four to forty-eight hours of washing in running water. The mounted and stained sections show shrinkage but it is uniform and therefore hard to detect. The acetone fixed sections that have been stained with colloidal silver show all the advantages revealed by the Bodian method and fixative. It preserves the most delicate and finest structures of the central nervous system, whether these elements are in a normal state or in a state of progressive degeneration. All degrees of degeneration (for which reason the technique was developed) as described by Ramon y Cajal (1928) are observed in the Zenker and acetone fixed material. In the acetone fixed tissue, the perikarya show up as brown colored structures with black neurofibrils coursing through. The nucleus is of a lighter brown

color and therefore its relative position within the cell can easily be studied.

Final Method.— In the hypothalamus, where the neurofibrils are mostly all unmyelinated, the degenerative changes within them are more pronounced for the same time element in contrast to myelinated fibers. Therefore, it was found advisable to perfuse the animal (cat) through the heart with the desired fixative (acetone or Zenker acetic) and therefore bring about a certain amount of brain tissue fixation and thus inhibit to a minimum post-mortem degeneration. On a normal cat of 5 Kilos, with descending thoracic aorta clamped, 100 to 200 cc. of fixative would be used. This amount has been found enough in order to inhibit unwanted degeneration to a minimum if at all.

Fixation: The brain is removed and placed in pure, unused acetone at low temperatures for forty-eight hours with a change after the first twenty-four hours or in modified Zenker acetic (Potassium dichromate, 3.5%; Mercuric chloride, 2 gr.; Sodium sulphate, 2 gr.; Glacial acetic acid, 5 cc.) for forty-eight hours with two changes.

Washing: Twenty-four to forty-eight hours. Often, the smell of acetone persists for many days, even after the brain has been dehydrated through graded alcohols. This fact is not a drawback for it has no ill effect on the staining.

Dehydration: Use graded alcohols beginning with 50% and ending with 2 changes of absolute. If a magnet mixer is used, 12 hours for each graded alcohol is sufficient. We use the alcohols over and over and discard them only when a cloudiness appears.

Clearing: Two changes of benzene, six hours each.

Note: The dehydration, clearing and embedding can very well be done by the dioxane method. No ill effect is observed. Though the dioxane method is time saving, it tends to make the tissues brittle and therefore difficult to cut. Furthermore, dioxane fumes are extremely toxic.

An alternative method: one to two cm. pieces of acetone fixed tissue can be placed directly into pure paraffin (or parawax) for twenty-four to forty-eight hours. The penetration is relatively slow but the end results are equally good.

Embedding: Benzene-paraffin for 6 hours.
Paraffin 1 " 6 "
" 2 " 6 "
" 3 " 6 "

The block is subsequently cut at any desired thickness. Our choice is 20 micra.

Deceration: Xylol - 1 — 2 minutes
Xylol - 2 — 2 "
Absolute alcohol - 1 — 2 "
" " - 2 — 2 "

Staining: 1. Treat the sections with absolute alcohol plus 1% of concentrated ammonia. Higher concentrations of ammonia (up to 5%) have been tried with equally good results. With higher percentages of ammonia there is a tendency for the tissue to shrink excessively and therefore to become detached from the slides. The tissue should remain in the above solution for six hours or more. No ill effect has been observed with tissues that have remained in alcohol-ammonia solution for twenty-four hours.

2. Wash the sections in 6 to 10 changes of distilled water. It is important that all the ammonia be removed.

3. Place into chemically pure unused pyridine (Fisher) for 6 hours or longer. If exposed for a longer period in pyridine (24 hours or more), the constituents of the cell body with the nucleus are entirely lost. Furthermore, there is a tendency for the neurofibrils to swell and their delicate make-up is lost. With prolonged pyridine treatment, the authors have never been able to find, within the central nervous system, the nodes of Ranvier which are otherwise seen (Bodian, 1952).

Experiments carried out on the possibility of eliminating the alcohol-ammonia and pyridine treatment have given conclusive results.

For a criterion of differential impregnation the cortex and a degenerated thalamic region have been used: the former for details of neurofibrils and the latter for terminal (free and bouton like endings) degeneration.

Serial paraffin sections from acetone fixed material were used in the following manner:

a) one set of slides were treated directly with colloidal silver nitrate without a previous treatment with alcoholic-ammonia solution or pyridine.

b) one set (with a difference of 20 micra for each section) was treated with alcoholic-ammonia solution without pyridine before silver impregnation.

c) one set, of the same region, was acted upon by pyridine but not alcoholic-ammonia solution and impregnated with the colloidal silver.

d) another set was treated with alcoholic-ammonia solution and pyridine before impregnation with colloidal silver.

The four sets of slides were passed through the various stages of the technique together, thus eliminating all possible sources of error.

The following results have been obtained:

(a) set one above:

1. background, light red.
2. heavy precipitate of colloidal silver at region of degeneration (thalamus due to electrolytic lesion).
3. absence of visible degenerative changes.
4. fibers appear thicker than normal.
5. contours of cell body are lost and neurofibrillar background is obscured.
6. nucleus and nucleolus partially stained.
7. neurofibrils appear "lifted" and broken.
8. no differential impregnation of non-myelinated fibers as seen at region of hypothalamus.

(b) Alcohol-ammonia treated sections.

1. background shows greater differentiation and less homogeneous. Pinkish in color.
2. greater differential impregnation of cell body.
3. myelinated fibers stand out most profoundly.
4. no differential impregnation of non-myelinated fibers (hypothalamus) and/or terminals (thalamus).

5. no apparent "lifting" of fibrils.

(c) Pyridine treated sections:

1. background is colorless.
2. better differential impregnation of myelinated and unmyelinated fibers.
3. cell contours are easily recognized.
4. nucleus unstained while nucleolus is lightly stained.
5. no heavy precipitate at region of degeneration.
6. terminals are to be observed at region of degeneration.
7. fibers are not "lifted" from the colorless background.

(d) as adopted in final technique.

From the above data, it becomes apparent that alcohol-ammonia and pyridine treatment should be used as a preliminary step before impregnating with colloidal silver. Pyridine is a fat solvent. Alcohol dissolves myelin and ammonia prevents shrinkage. It appears that the above chemical solutions bring about a favourable physiological reaction which predisposes the neurofibrils to silver impregnation and greater differentiation especially for the detection of terminal degeneration.

4. Wash the section in distilled water, 6 to 10 changes. The washing is necessary to remove the excess pyridine. Colloidal silver nitrate in the presence of pyridine will not give the desired differentiation though the impregnation is as good.

5. Sections are placed in 2% silver nitrate overnight at 28-30°C. Equally excellent preparations have been obtained at a temperature of

60° C. for 6 hours.

At temperatures of 28-30° C., it is recommended that 15-17 hours of impregnation be the maximum. With 24 hours of impregnation, excess deposition of colloidal silver begins to take place and therefore a partial loss of differential impregnation.

Ordinarily, 10 slides with 10 sections are impregnated in approximately 280-290 cc. of 2% silver nitrate. The used colloidal silver is discarded.

6. The sections are given a quick dip in absolute alcohol, few seconds, and immediately placed in the reducing agents. With each batch of 10 slides, the absolute alcohol is discarded. The alcohol serves the purpose of removing the excess colloidal silver that is present on the sections and is not a critical factor in the technique.

Reducing substances were tried in various concentrations, either alone or in combination with other substances. These included hydroquinone, pyrogallic acid, tannic acid, alcohol, oxalic acid, and formaldehyde. The tissues mostly used for this test were acetone, alcohol-formaldehyde, and modified Zenker acetic fixed material.

The following combinations and proportions were used. (The relative degree of effectiveness is indicated with + and - signs.)

Substances		Solutions		
		acetone	alcohol-formol	Zenker
Hydroquinone	.1%	-	-	-
	.5%	- +	- +	-
	1 %	+ +	+ +	+ +
	2 %	+	-	- -

Substances		Solutions: acetone	alcohol-formol	Zenker
	5 %	-	-	-
	10 %	-	-	-
Pyrogallic acid	.1%	-	-	-
	.5%	-	-	-
	1 %	-	-	+
	2 %	+	+	+
	5 %	+	+	-
	10 %	-	-	-
Tannic acid	.1%	-	-	-
	.5%	-	-	-
	1 %	-	-	-
	2 %	-	-	-
	5 %	+ -	-	-
	10 %	-	-	-
Alcohol	75 %	-	-	-
	95 %	- +	- +	- +
	100 %	-	-	-
Oxalic acid	.1%	-	-	-
	.5%	-	-	-
	1 %	+ +	+ +	+ +
	2 %	+	+	+
	5 %	-	-	-
	10 %	-	-	-
Formaldehyde (commercial, non-neutralized formalin taken as 100 %)	1 %	-	-	-
	2 %	-	-	-

Substances		Solutions acetone	alcohol-formol	Zenker
	3 %	-	- +	-
	4 %	+	+	+
	5 %	+ +	+ +	+ +
	10 %	-	- +	-

Combinations of solutions included the following with proportions and degrees of effectiveness.

		acetone	alcohol-formol	Zenker
Hydroquinone	1 gr.			
Pyrogallic acid	2 gr.	+ +	+ +	+
95% alcohol	95 cc.			
Formaldehyde	4 cc.			
Hydroquinone	2 gr.			
Pyrogallic acid	5 gr.	+ +	+ +	+
95% alcohol	95 cc.			
Formaldehyde	5 cc.			
Hydroquinone	1 gr.			
95% alcohol	95 cc.	+ +	+	+
Formaldehyde	4 cc.			
Hydroquinone	2 gr.			
95% alcohol	95 cc.	+ + +	+ +	+ +
Formaldehyde	5 cc.			
Pyrogallic acid	2 gr.			
95% alcohol	95 cc.	+ + +	+ +	+ +
Formaldehyde	7 cc.			
Pyrogallic acid	5 gr.			
95% alcohol	95 cc.	+ + +	+ +	+ +
Formaldehyde	5 cc.			
Pyrogallic acid	3 gr.			
95% alcohol	95 cc.	+ + + +	+ + +	+ + +
Formaldehyde	5 cc.			

The last formula consistently gave the best result and therefore it was adopted within the final technique.

The scheme mentioned above was also used for other tissues which had been fixed in the other fixatives but none of them approached the results obtained with acetone, alcohol-formol and modified Zenker acetic fixed tissues.

The intensity of the stain after this first reduction is found to be sufficiently contrasting to allow detailed studies on normal tissues.

7. The reductor is made up of:

95% alcohol	95 cc.
Analytical formalin	5 cc.
Pyrogallic acid	3 gr.

and the tissues are left in this solution for 4 minutes at which time the staining rack with the slides is constantly agitated. Pyrogallic acid is more soluble in alcohol (95%). Therefore, it is advisable that the pyrogallol be dissolved in the alcohol first and the formalin be added to the solution. The slides become yellow and the solution black. The solution is never used more than once as it loses its reducing power with 10 slides.

If there is no need for the study of the delicate morphological terminal degenerating changes, or for the study of normally present minute and delicate structures in the central nervous system, the amount of formalin can be increased to 8 cc. in the reducing solution which blackens the neurofibrils. In that case there is no necessity to further intensify in the toning process with gold chloride. Under these circumstances, the sections are washed thoroughly, fixed in 10% sodium-thio-sulphate, washed thoroughly again in distilled water, dehydrated, cleared and covered accordingly.

8. Dip in two changes of 50% alcohol. This step is advisable

in order to remove completely the constituent elements of the reductor. Distilled water can be substituted for the two alcohols but it has been found that the sudden change from 95% alcohol in the reductor to water caused the sections to be lifted from the slides.

9. A thorough washing in distilled water. Six to ten changes are advisable to remove all the constituents of the reductor as well as the alcohol.

10. Tone with 1% yellow gold chloride to which three drops of glacial acetic acid has been added. The toning process lasts only a few seconds. When the reduction reaction is completed, $3\ Ag + AuCl_3 = 3\ AgCl + Au$, the tissue is totally colorless. Gold concentrations of .1 to .8% have been found as efficient as 1%. The concentration of gold chloride is uncritical except that the more concentrated solutions toned faster than dilute ones. Acetic gold chloride has been found superfluous by Davenport and Kline (1938) which is contrary to our findings but in accordance with Bodian (1936). Basic and neutral gold chloride has been found useless as it does not bring about the above chemical reaction. Therefore, it is apparent that hydrogen ions are the critical factors which swing the equilibrium constant to the right in favor of gold intensification. Neutral gold chloride was found most critical in the preparation of the Golgi type of stain for serial paraffin sections which is to be reported at a later communication (see plates).

For better contrast, in order to bring out the finest structures, normal as well as pathological, a second reduction after gold toning has been found necessary. Gold toning is an extremely effective means of

increasing the intensity of the stain. With this procedure, it was shown that the concentration of gold chloride used in toning was faster with greater concentrations and that it was critical for the finer structures. Therefore a 1% gold chloride solution was ordinarily used. Customarily, three drops of glacial acetic acid is added to the yellow gold solution. The final colloidal gold solution has an approximate pH 4.

When the reaction $3Ag + AuCl_3 = 3AgCl + Au$ was completed, as shown by the pale light colour of the section, the excess gold chloride is thoroughly washed off with distilled water.

11. Six to ten changes of distilled water are recommended. The silver chloride which remained in the tissue is further reduced by the 1% oxalic bath. Since this second reduction brings out the finer details, it is important that the time variation be maintained to 3½ minutes within the oxalic bath, at which interval, the slide rack is shaken constantly. During the course of removing variable factors, one with the oxalic bath, it was found that if the tissue is left in the oxalic bath less than 3½ minutes, the finer details of the finest neurofibrils do not contrast well with the background. On the other hand, if the oxalic bath acts on the tissues for a period more than 3½ minutes non-nervous structures tend to develop and no marked improvement in the nervous tissue is gained by prolonging the oxalic acid treatment.

A series of controls were undertaken to determine which of the reductors at hand worked most efficiently in bringing out the finest of details.

The following reagents were used, at various concentrations,

diverse combinations and for different time intervals. It included hydroquinone, pyrogallic acid, tannic acid, alcohol, formaldehyde and oxalic acid. (The relative degree of effectiveness is indicated with + and - signs as observed on acetone and Zenker acetic fixed tissue.)

Substance	.5%	1.0%	1.5%	2.0%	3.0%	5.0%
		Concentrations for 1 minute				
Hydroquinone	-	-	-	-	-	-
Pyrogallic acid	-	-	-	-	-	-
Tannic acid	-	-	-	-	-	-
Oxalic acid	-	-	-	-	-	-
		Concentrations for 2 minutes				
Hydroquinone	-	-	-	-	-	-+
Pyrogallic acid	-	-	-	-	-	-
Tannic acid	-	-	-	-	-	-
Oxalic acid	-	-	-	-	-	-+
		Concentrations for 3 minutes				
Hydroquinone	-	+++	++	+	+	+
Pyrogallic acid	-	+-	++	++	+++	+-
Tannic acid	-	+-	+-	+-	+-	+-
Oxalic acid	-	++++	+++	+++	+-	+-
		Concentrations for 4 minutes				
Hydroquinone	+	++	++	++	++	+-
Pyrogallic acid	+-	+++	+++	+++	+++	++
Tannic acid	+-	+-	+-	+-	+-	+-
Oxalic acid	++++	++++	++++	+++	++	+-

Substance	Concentrations for 5 minutes					
	.5%	1.0%	1.5%	2.0%	3.0%	5.0%
Hydroquinone	+	++	+-	-	-	-
Pyrogallic acid	+	++	+-	-	-	-
Tannic acid	+	+-	+-	-	-	-
Oxalic acid	++	+-	+-	+-	+-	-

	Time in Minutes					
Alcohol	.5 Min.	1 Min.	2 Min.	3 Min.	4 Min.	5 Min.
50%	-	-	-	-	-	-
75%	-	-	-	-	-	-
95%	-	-	-	+-	+-	+-
100%	-	-	-	-	-	-
Formaldehyde (Commercial, non-neutralized formalin. Taken as 100%)						
5%	-	-	-	+-	+-	+
10%	-	-	+-	+	+	+
15%	-	-	+-	+	+-	-
20%	-	-	-	-	-	-

Combinations of the above solutions with proportions, time factor and degree of effectiveness. The latter is indicated with + and - signs.

		Minutes					
		.5 Min.	1.0 Min.	2 Min.	3 Min.	4 Min.	5 Min.
Hydroquinone	1 gr.						
Pyrogallic acid	2 gr.						
95% alcohol	95 cc.	-	-	-	+-	+	+
Formaldehyde	4 cc.						

		Minutes					
		.5 Min.	1.0 Min.	2 Min.	3 Min.	4 Min.	5 Min.
Hydroquinone	2 gr.						
Pyrogallic acid	5 gr.						
95% alcohol	95 cc.	-	-	-	-	+-	-
Formaldehyde	5 cc.						
Hydroquinone	1 gr.						
95% alcohol	95 cc.	-	-	-+	-+	+-	+-
Formaldehyde	4 cc.						
Hydroquinone	2 gr.						
95% alcohol	95 cc.	-	-	-	+-	+	+-
Formaldehyde	5 cc.						
Pyrogallic acid	2 gr.						
95% alcohol	95 cc.	-	-	-	+	+	+-
Formaldehyde	7 cc.						
Pyrogallic acid	5 gr.						
95% alcohol	95 cc.	-	-	-	+	+	+
Formaldehyde	5 cc.						
Pyrogallic acid	5 gr.						
95% alcohol	95 cc.	-	-	-	+	+	+-
Formaldehyde	5 cc.						

For a specific time element, except for alcohol, all of above reagents appear to be sufficiently effective to bring about reduction of colloidal silver, but only oxalic acid was able to give a selective differential reduction.

12. Again wash well in distilled water, 6 to 10 changes to remove the oxalic acid on the sections.

13. Treat with 10% sodium-thio-sulphate for 5 to 10 minutes to fix the silver. High concentration of sodium-thio-sulphate has been found most valuable in control tests. It is true that some reduced silver is removed as observed on Golgi preparations but the end result is better with high concentration than with lower percentages of sodium-

thio-sulphate. Whether this is the result of pH factor or the sulphate cannot be decided. With the use of sodium sulphite, which is less effective than the sulphate, Holmes (1943), in contrast to Silver (1942), is of the opinion that the pH does not materially affect the stain. Room temperature has been found significantly high (25° C.) to cause an effective development.

14. Wash thoroughly with distilled water, 6 to 10 changes.

15. Dehydrate with graded alcohols and in 2 changes of Xylol and coverslip in the usual way with Canada balsam or synthetic balsam.

Outline of Final Technique

Fixation: Whole brains (cat) are fixed in cold acetone for forty-eight hours or longer with a change once after the first twenty-four hours, or in modified Zenker acetic for seventy-two hours with 2 changes after the first and second twenty-fourth hour.

Washing: Twenty-four hours in running water. If the smell of acetone is still noticeable the washing should be continued for another twenty-four hours.

Dehydration: graded alcohols. 6 hours each in Mag. Mix.

Clearing: two changes of benzene. 6 hours each.

Embedding: benzene - paraffin - 6 hours

paraffin 1. - 6 hours

paraffin 2. - 6 hours

paraffin 3. - 6 hours

The block may subsequently be cut in sections of 15 to 20 micra.

Deparaffinizing: xylol 1. - 2 minutes

xylol 2. - 2 minutes

100% alcohol 1. - 2 minutes

100% alcohol 2. - 2 minutes

Technique proper:

1. 100% alcohol + 1% conc. ammonia - 6 hours or longer.
2. Wash in distilled water - 6 to 10 changes.
3. Pure pyridine - 6 hours or longer.
4. Wash in distilled water - 6 to 10 changes.
5. 2% silver nitrate - overnight or longer at 30° C.
6. Dip in absolute alcohol - to remove excess silver.
7. 4 minutes in: 95 cc. of 95% alcohol
   5 cc. of analytical formalin - agitate at intervals.
8. Dip - 50% alcohol 1)
   50% alcohol 2)
9. Wash in distilled water - 6 to 10 changes.
10. Tone with 1% gold chloride + 3 drops of glacial acetic acid per 100 cc. till sections are totally decolorized. Few seconds.
11. Wash in distilled water - 6 to 8 changes.
12. 1% oxalic acid - 3½ minutes.
13. Wash in distilled water - 6 to 8 changes.
14. 10% sodium-thiosulphate - 5 to 10 minutes.
15. Dehydrate - graded alcohols.
16. Clear in xylol - 2 changes.
17. Coverslip.

Discussion on the dynamics of silver staining.— Cajal (1903) believed that tissues contained albumen which united with silver and that the resulting silver compounds could be easily reduced, thus making structures visible. In 1920, without data, Cajal made the suggestion that in silver-ammino-carbonate solutions an "ammoniacal oxide" of silver is produced which is taken up by the histological elements in a selective manner. The view presented by Bolsi (1927) was essentially an elaboration of Cajal's idea. The latter believed that the silver salts are selectively absorbed by tissue elements.

That the Cajal stain is analogous to the photographic process was theorized upon by Liesegang (1911). The latter author maintains that while the tissue is in colloidal silver nitrate solution, by virtue of an inherent reducing action of the histological elements, some silver is reduced which forms the nuclei as in the exposed photographic plate. Through further treatment with hydroquinone these nuclei have silver deposited on them and therefore an outline of the histological elements.

Liesegang's theory was further elaborated by Cajal (1921). The latter accepted the "deposition" principle on the character of the stain but offered two objections to the idea of a first formed nucleus. The first objection based on experimental data was that the treatment of tissues with oxidizing agents did not change the staining, and the second, that the theory did not explain what he called the "transference of nucleation". Therefore, as Cajal pointed out, when tissues were treated with various reagents, different structures were stained.

Kubie (1929) suggested that when tissues are placed in the

reducing bath following immersion in silver-ammino-solutions, silver salts tend to diffuse out and, depending upon the speed of reduction, silver will be deposited either in the tissue or upon its surface.

The reduction reaction is thought to form a silver salt (Visintini, 1931) and that this salt is then specifically flocculated upon histological elements.

Zon (1936) has conclusively shown that the simple views advocated by Cajal and Bolsi must be altered. Zon maintains that the deposition of colloidal silver must play a part in staining and that the deposition depends upon the rate at which the reduction reaction occurs on and within histological structures.

The presence of reducing substances in tissues which might form catalytic "nuclei" of silver has been demonstrated by Loew and Bokorny (1881), Masson (1928), Kon (1933) and others.

If such "nuclei", submicroscopic particles of either metallic silver or a silver compound, were formed, it might be thought that it would be easy to manipulate them by means of the well known procedures of photography and thus determine their influence. Cajal pointed out that such a manipulation is inconclusive. It might be added from our observations that if such nuclei are formed in the tissues, they must be highly protected by the protoplasmic gel structures and are therefore of secondary importance.

Zon's (1936) elaboration on the gel theory assumes that the nerve fibers stain easily with silver because their gel structure allows precipitation of metallic silver to occur readily. Nageotti and Gyon (1930) substantiate Zon's observations. The above authors have shown

by observations with the cardioid condenser that silver stained structures contain colloidal particles of silver.

Von Weimann, as quoted by Gortner (1929), has shown that the degree of dispersion of a sol is dependent upon the ratio of the rate of nuclear formation to the rate of crystal growth. The above theory would account for the color difference in nerve fibers stained with silver. The conception of the Golgi stain, according to Zon, based upon the above considerations is as follows:

The brain substances form a non-homogeneous gel. When the tissue is immersed in potassium dichromate it takes up a certain amount of the chromium salt. The silver nitrate diffusing in produces a supersaturated solution of silver dichromate. Precipitation begins in the regions where protective power is least. More silver dichromate diffuses to those regions and is added to the precipitate already present.

From the observations on Golgi preparations as prepared in bulk and serial paraffin sections, we are inclined to believe that Zon's idea that precipitation begins in regions where protective power is least is well conceived as it would explain the selectivity of specific neurons at specific regions (Golgi stain). But Zon's theory does not explain, with respect to Golgi preparations, why under controlled conditions, the same specific neurons of the same specific area are not impregnated.

Zon makes no attempt to explain why under the same specific conditions, at the same region, neurons have degrees of impregnations. The theory is upheld by his Golgi impregnation method on the liver. The bile canaliculi stand out sharply. The lumen, presumably being spaces, would fill up with precipitated silver dichromate, while the cells being protective gels would have only a finely dispersed precipitate. This

would also be an explanation on the frequency in which capillaries stand out most elaborately with Golgi type of stain. Careful examination of blood capillaries in our preparations shows that the structures seen are not solid rods of precipitated silver dichromate.

Visintini (1931) holds that the particle size does not determine what structures are stained. Zon believes that the structures determine by their properties the size of the colloidal particle produced within them. If such were the case of the dynamic phenomena of silver staining, all neurons of specific region should be impregnated equally. The capriciousness of the Golgi type of stain would eliminate such a simple explanation.

Though fixatives, in part, appear to change the protective power of the gels and also the ability to absorb silver, it still does not explain the capriciousness of Golgi preparations. We have proven (to be reported in a later communication) that fixation and tissue predisposition and therefore variability in tissue substratum is indeed the most important variable in silver staining which is contrary to the observations of authors that worked with Cajal and Golgi types of stains.

From the presented evidences, it becomes apparent that all nervous structures, central as well as peripheral, have the same affinity for silver and therefore the protective power of the gel is the same in all regions. This is contrary to Zon's conclusion. We believe that the fundamental constituents of different neurons from different regions which subserve different functions are not alike in essence, but can be made similar as a predisposition to silver staining if treated with

the proper solutions, for instance, alcohol-ammonia and pyridine as described in the presented technique, and bromine water for the Golgi stain.

CHAPTER III

OBSERVATIONS

Material.— A group of 32 cats has been used for experiments with localized lesion. In all instances, sterile technique and nembutal anesthesia (20 mg./Kilo of body weight) were used. The anesthetic was injected intraperitoneally.

The cats were firmly oriented in the Horsley-Clarke stereotaxic apparatus. The coordinates of the apparatus defined the brain region to be exposed.

The skin, subcutaneous tissue and periosteum were incised in the median plane and reflected laterally.

The bone was trephined with a dental drill (for knife lesions) or with a hand trephine (1 inch in diameter) for electrolytic lesions. In all instances, the dura was incised to a minimum and whenever possible, it was sutured. The lesions were unilateral, though those placed close to midline had a tendency, due to irradiation, to overlap. Bone wax was employed to replace the removed bone and the periosteum and skin was approximated and sutured with silk thread while for the skin, nickel wound clips were used.

The cats were allowed a variable survival time which ranged from 4 days to 14 days. The majority was sacrificed on the 4th or 5th post-operative day.

While under deep anesthesia, the thorax was exposed and the right atrium incised. The thoracic aorta was clamped and 200 to 300 cc. of fixing fluid was introduced through the left ventricle.

The brain, including the pituitary, was removed immediately with the least possible handling of the brain tissue. This procedure normally took 10 to 15 minutes. While removing it, the brain was constantly bathed with fixing fluid in order to prevent drying of the surface. This last step has been found most desirable as it inhibits the action of air on the tissue to a minimum. If the tissue is allowed to dry, the cortical areas will not stain well with colloidal silver (layer I and II).

The whole brain was then immersed in fixing fluid (ratio of fluid to brain volume is approximately 30 to 1). If acetone was used as the fixative, the brain (with dura removed) was allowed a period of 48 hours in cold acetone or longer with changes every 24 hours. In the modified Zenker acetic fixative, 48 to 72 hours has been the minimum time and the fluid is changed also at every 24-hour period (see Chapter II).

Six cats were used of this group as most suitable for studies on the connections of the magnocellular nuclei. All the brains were serially sectioned at 20 micra thickness. Every 9th and 10th sections were mounted and respectively stained with toluidine blue for retrograde changes and silver for axonal and terminal degeneration.

Observations:

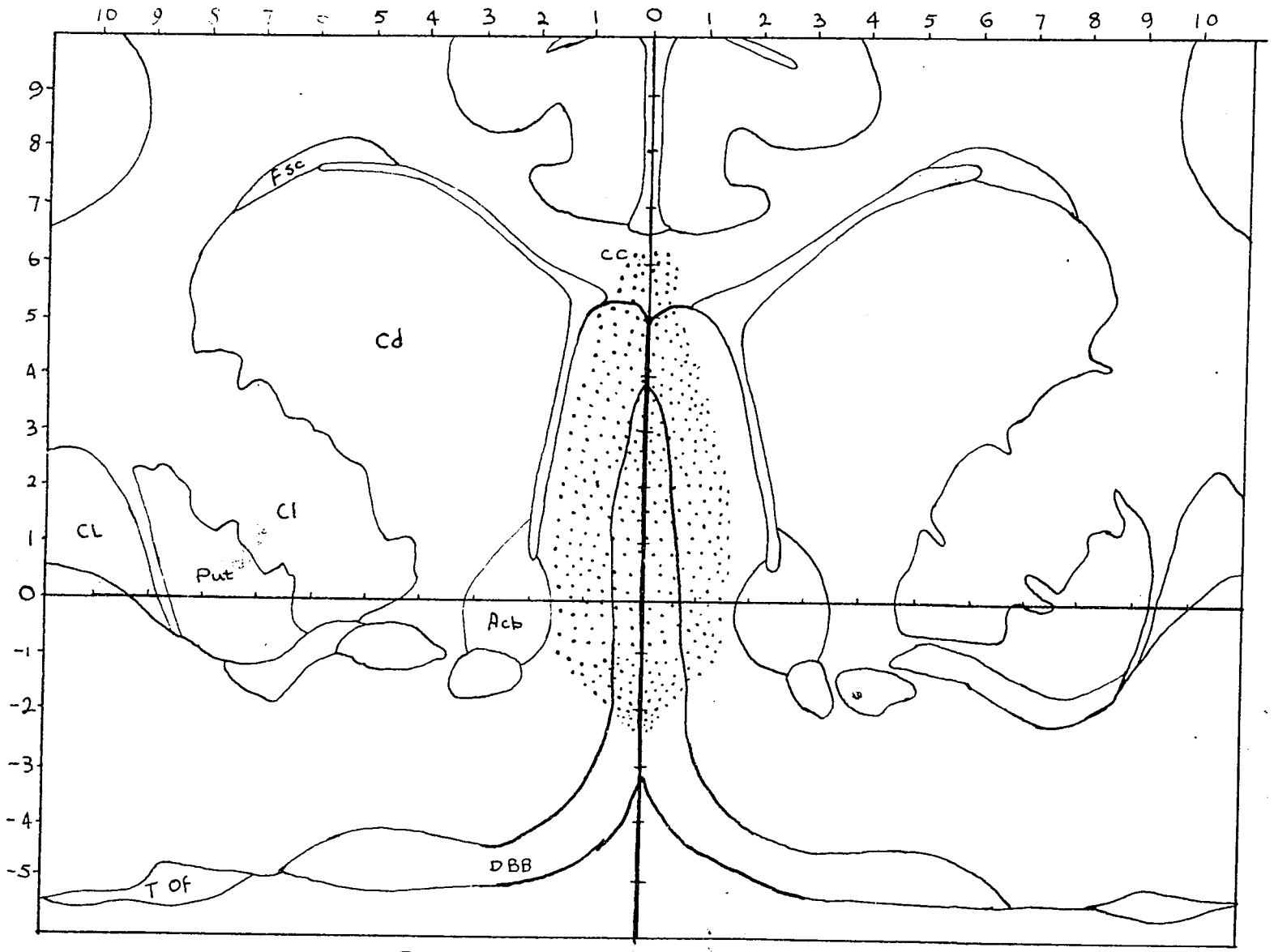
Cat - 1AZ - Post-operative period of two weeks.

- Perfused with 100 to 200 cc. of modified Zenker acetic and fixed in the above fixative for 3 days.
- Insertion of electrodes according to the Horsley-Clarke stereotaxic coordinates:

Frontal	17
Lateral	.5
Horizontal	+2, 3, 4, 5, 6

- Localization of lesion.-

The medial and lateral septal nuclei are completely destroyed. The dorsal-most part of the diagonal band of Broca and the corpus callosum (mid-line) have also been destroyed.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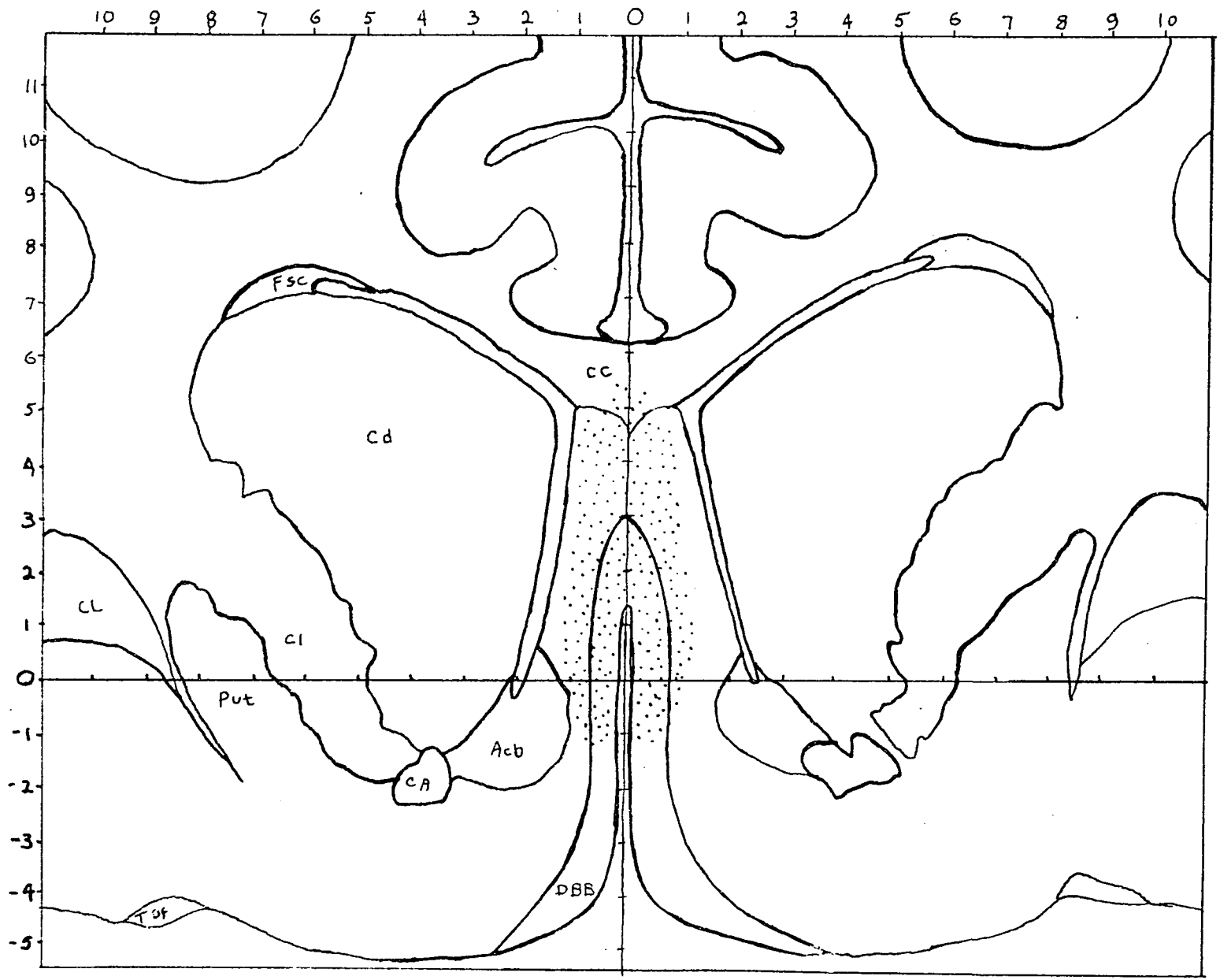
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- Histological examination.

Characteristic cellular and fibrillar reaction is observed in the paraventricular nucleus.

The paraventricular nucleus is subdivided into three major portions:

(1) An antero-lateral magnocellular portion.

(2) An antero-medial mixed portion making up the bulk, and

(3) A postero-medial small-celled portion.

The postero-medial small cell portion cannot be referred to as the periventricular portion because the cells differ markedly from the periventricular cells.

Observations (Cont'd.):

The cellular degenerative changes are observed only within the antero-medial mixed cellular portion, decidedly within the large-celled component. Within this group of cells, the fibrils have lost their fine texture and are coarse or broken up into thick rods. The nuclei show pyknotic qualities and the Nissl material shows degrees of granulation. Vacuolation is also apparent.

The two other portions of the nucleus show no change whatever.

Fibers along the paraventriculo-hypophysial tract show degenerative changes of the beading type.

No typical degenerative (boutons, rings or clubs) changes can be observed within the pars nervosa of the pituitary gland.

Observations:

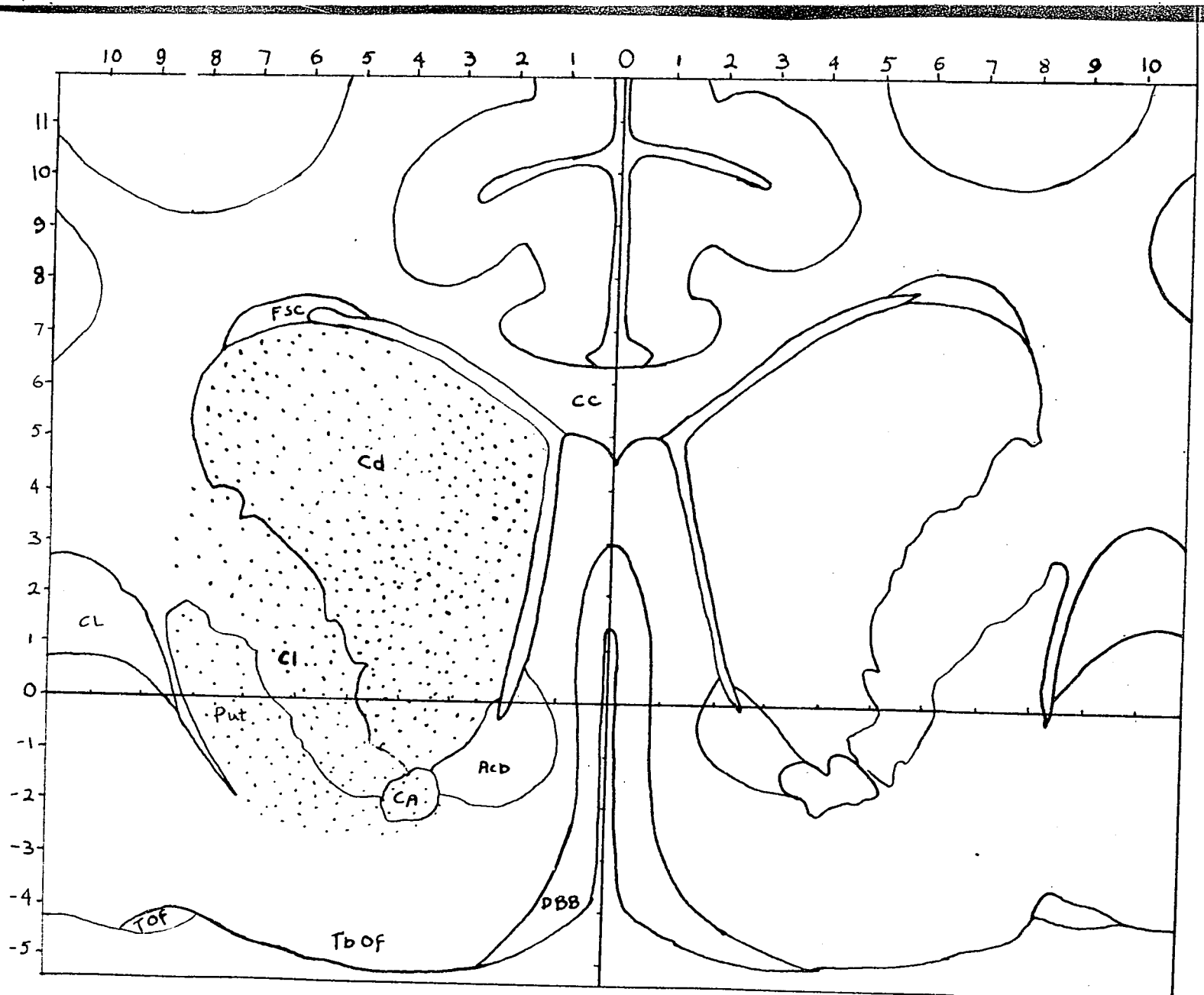
Cat 2A - Post-operative period of 4 days.

- Perfused with 100 to 200 cc. of cold acetone and fixed in above solution for 48 hours in cold with one change after the first 24 hours.
- Insertion of electrodes according to Horsley-Clarke stereotaxic coordinates:-

Frontal	17.0
Lateral	4
Horizontal	+4, +3, +2, +1, -1-2-3

- Localization of lesion:-

Complete destruction of head of caudate nucleus, anterior limb of internal capsule, and in part, the most anterior aspect of putamen, globus pollidus, amygdaloid complex, and the olfactory tubercle and cortex.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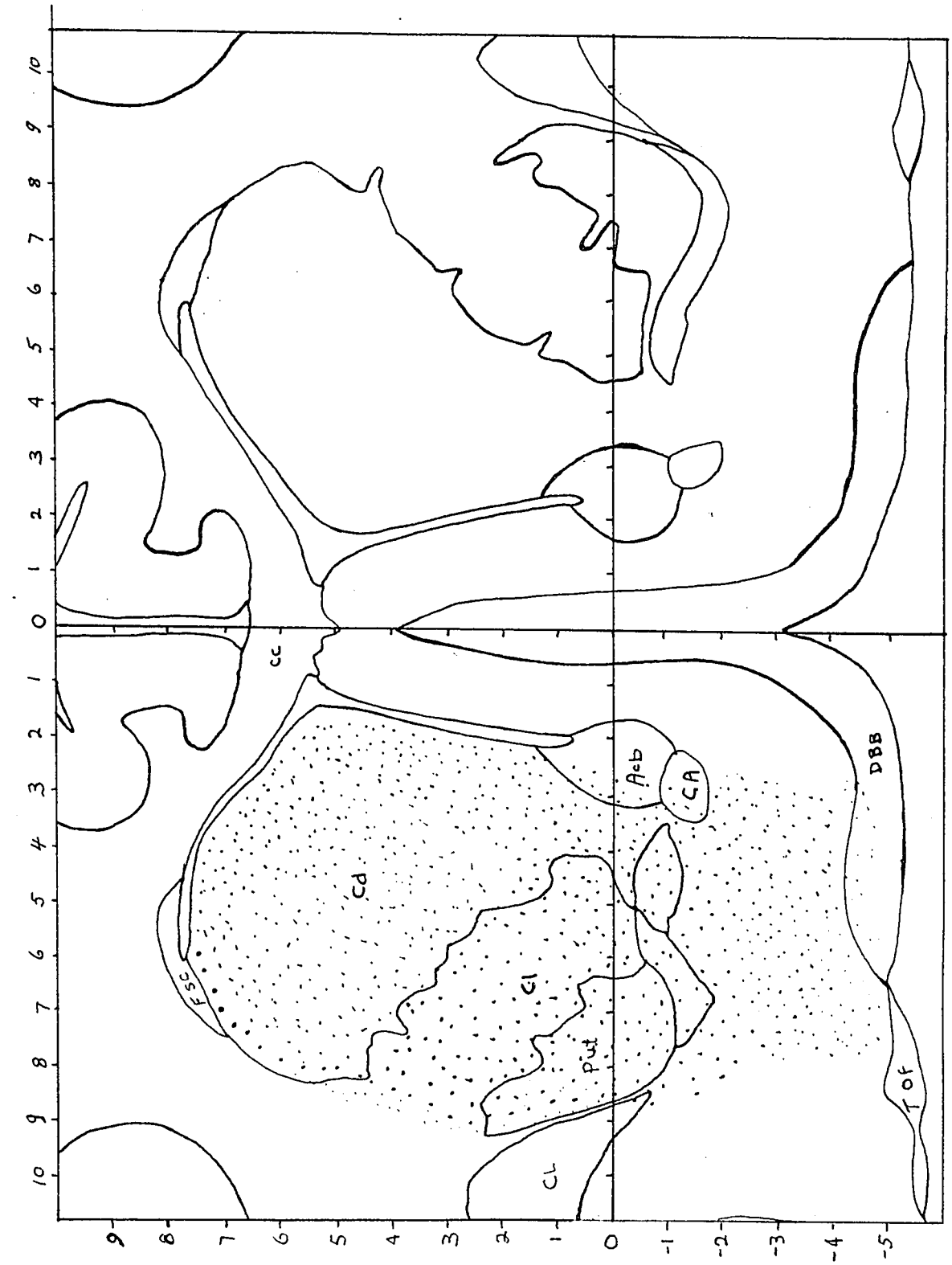

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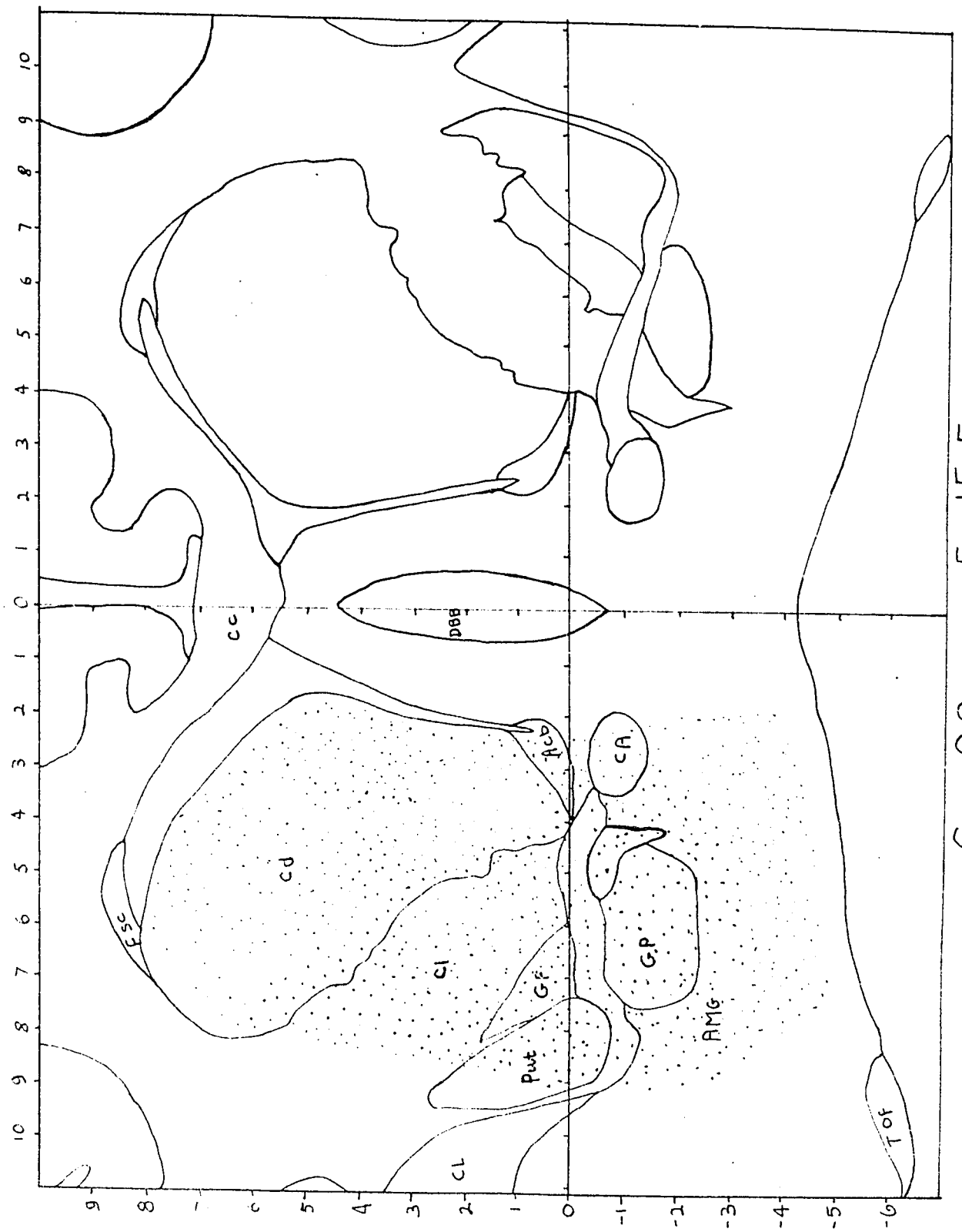
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- Histological examination.

Terminal boutons are found throughout the extensive medial and lateral hypothalamic area, most pronounced in the latter region. The ring type of boutons seems to predominate in these areas. To a lesser degree, scattered bulbs are also seen while the fibers show degrees of degenerative changes. The dorsal hypothalamic area is predominantly permeated by the free ending type of degeneration.

The paraventricular nucleus (the two major medial portions) and supraoptic nucleus, most pronounced in the latter, are permeated by ring-like endings. Equally extensively affected is the infundibular region, the suprachiasmatic and ventromedial hypothalamic nucleus.

Observations (Cont'd.):

The entopeduncular nucleus shows many ring-like terminals.

Of thalamic nuclei, bouton and free ending type of degeneration is observed in the dorso-medial, the same side as lesion, and in all haberular nuclei of the same and the opposite side of the lesion. The greatest distribution of terminals is to be found in the medial habenular nucleus of the same side as lesion.

The dorsomedial nucleus shows primarily fibrillar reaction with few scattered boutons among the neurites while the habenular nuclei show mostly the ring type of terminal degeneration. Of mid-line nuclei, the nucleus centralis pars medialis and lateralis receive the heaviest projection in the form of terminal rings. The nucleus rhomboidalis and reuniens sharing in various shapes of fibrillar reactions but with no decisive terminals. The anterior group of nuclei (antero-dorsal, antero-ventral and antero-medial) of same and opposite side of lesion show terminals of ring type.

No degenerative changes have been seen in the mammillary complex.

Observations

Cat 16A - Post-operative period of 7 days.

- Perfused with 100 to 200 cc. of formol-alcohol and fixed in above solution for one week.
- Insertion of electrodes according to Horsley-Clarke stereotaxic coordinates:-

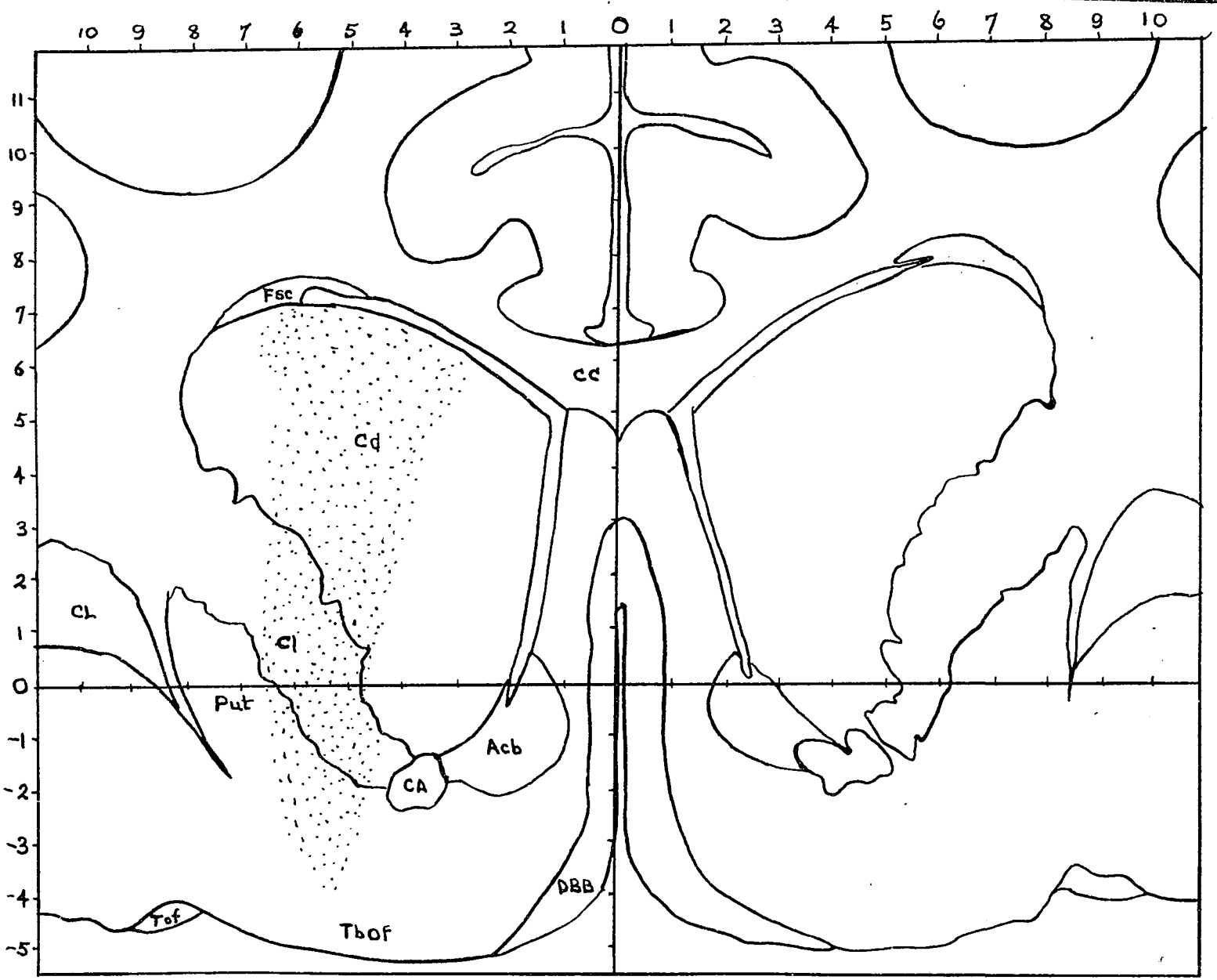
  Frontal 17.0

  Lateral 4

  Horizontal +5, +4, +3, +2, +1, -1

- Localization of lesion:-

  In part, head of caudate, anterior limb of internal capsule and most medial aspect of putamen.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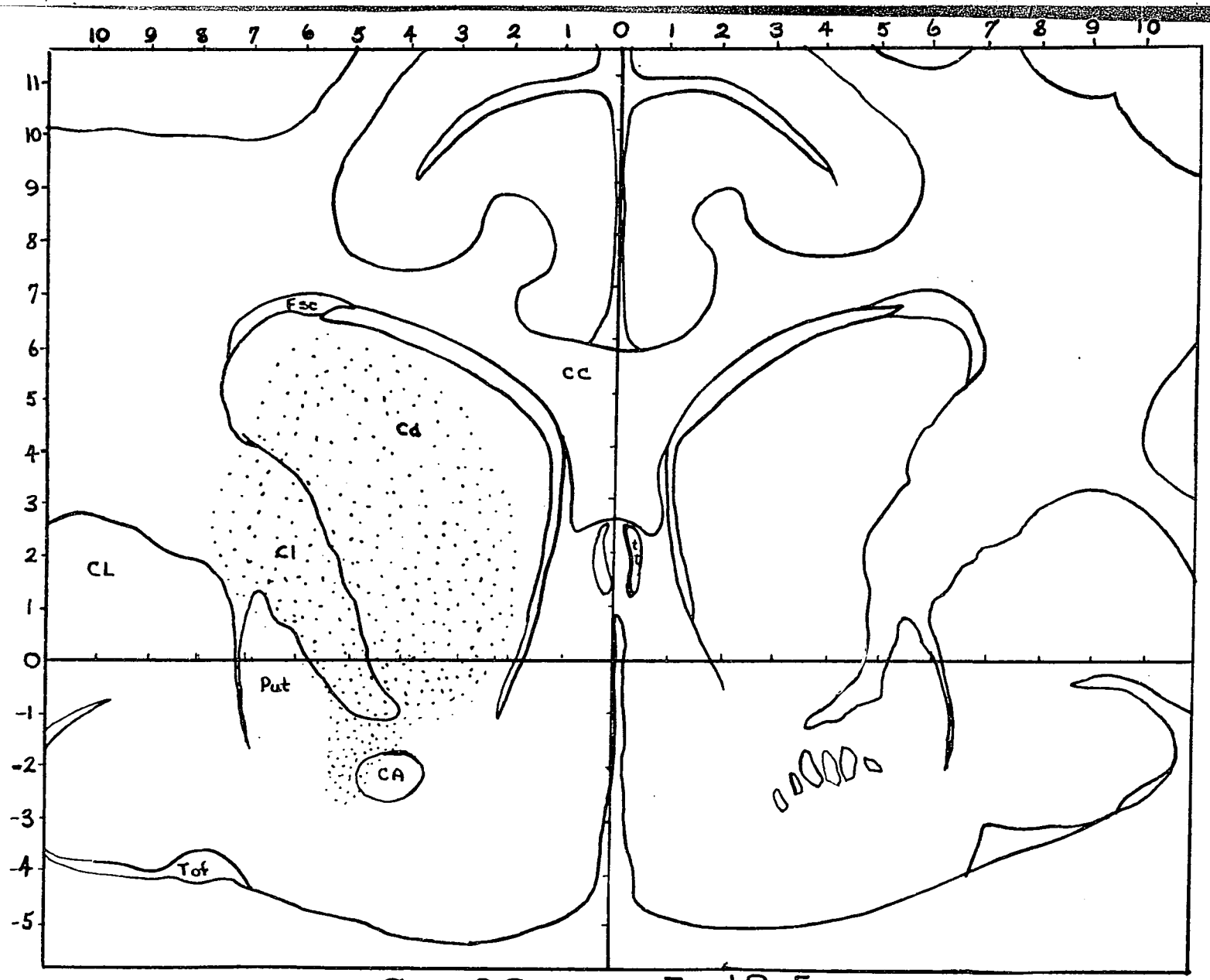
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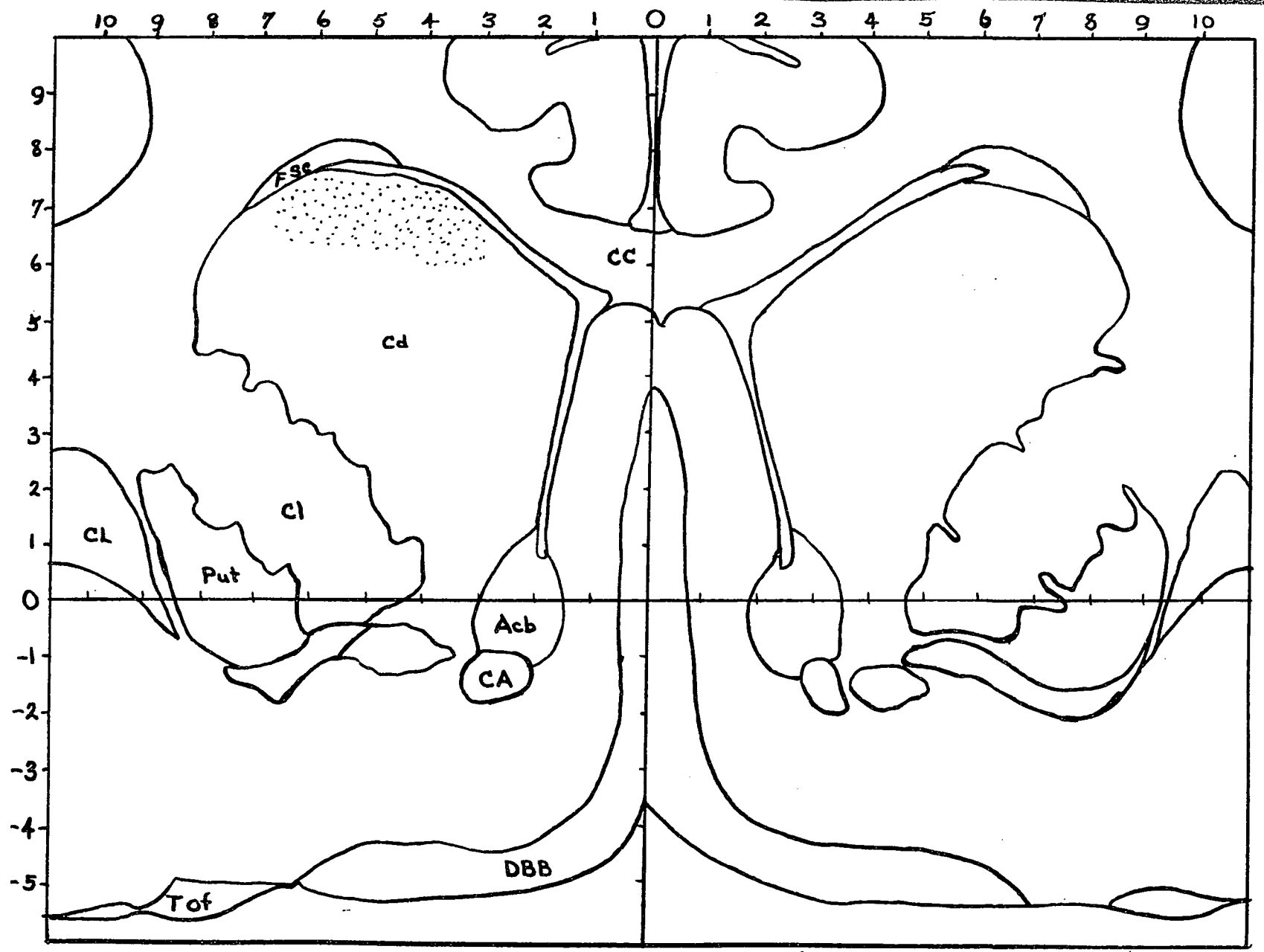
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Sec. 70 Fr. 16.0

- Histological examination.

Numerous terminals are seen within the medial and lateral preoptic area. The rings are fine and delicate. Swellings, tortuous and beaded fibers are also present.

The anterior portion of the supraoptic nucleus receives a particularly heavy projection. To a limited degree, indications of fibrillar reaction is to be seen within the paraventricular nucleus. No boutons have been found within this nucleus.

Beaded and broken fibrils enter the nucleus commissuralis interanteromedialis from the antero-lateral direction. These fibers do not cross to opposite side, but rather leave the main fasciculus and course ventrally to enter the nucleus reuniens and more posteriorly they enter the anterior portion of nucleus centralis medialis wherein these fibers end in the form of beading, fragmentation and scattered boutons of the ring type. The nucleus centralis pars lateralis is affected on same and opposite side of lesion.

The dorso-medial nucleus of thalamus on same side as lesion is predominated by axonal reaction wherein the neurofibrils are in the form of short, heavily stained rods. Few remaining boutons are also seen. On opposite side of lesion, the dorsomedial thalamic nucleus is permeated by terminal boutons accompanied by fibrillar reaction.

The centrum medianum on same side as lesion show terminal rings, beading and vacuolization. The amount of boutons in the centrum medianum per unit area as compared with nucleus dorso-

medialis, both on same side as lesion, far exceeds in number those in dorsomedial nucleus.

No indications of terminal degeneration is to be found in the nucleus ventralis postero lateralis.

Observations:

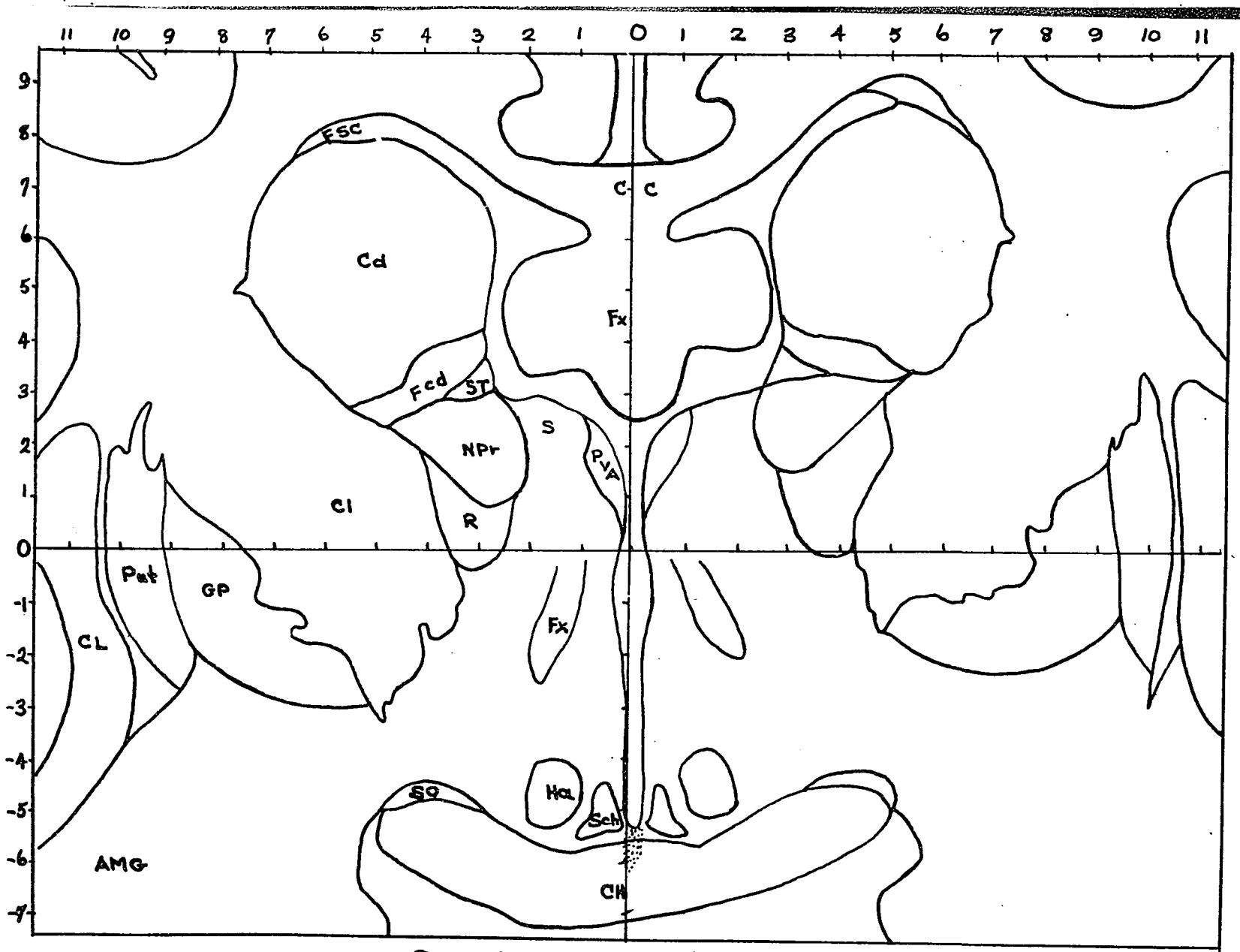
Cat 26A - Post-operative period of 4 days.

- Perfused with 100 to 200 cc. of cold acetone and fixed in above solution for 48 hours in cold with one change after the first 24 hours.
- Insertion of knife according to Horsley-Clarke stereotaxic coordinates:-

Frontal	14.0 to 10.5
Lateral	Midline
Horizontal	-4 to -7

- Localization of lesion:-

  Mid-line of the infundibular area.



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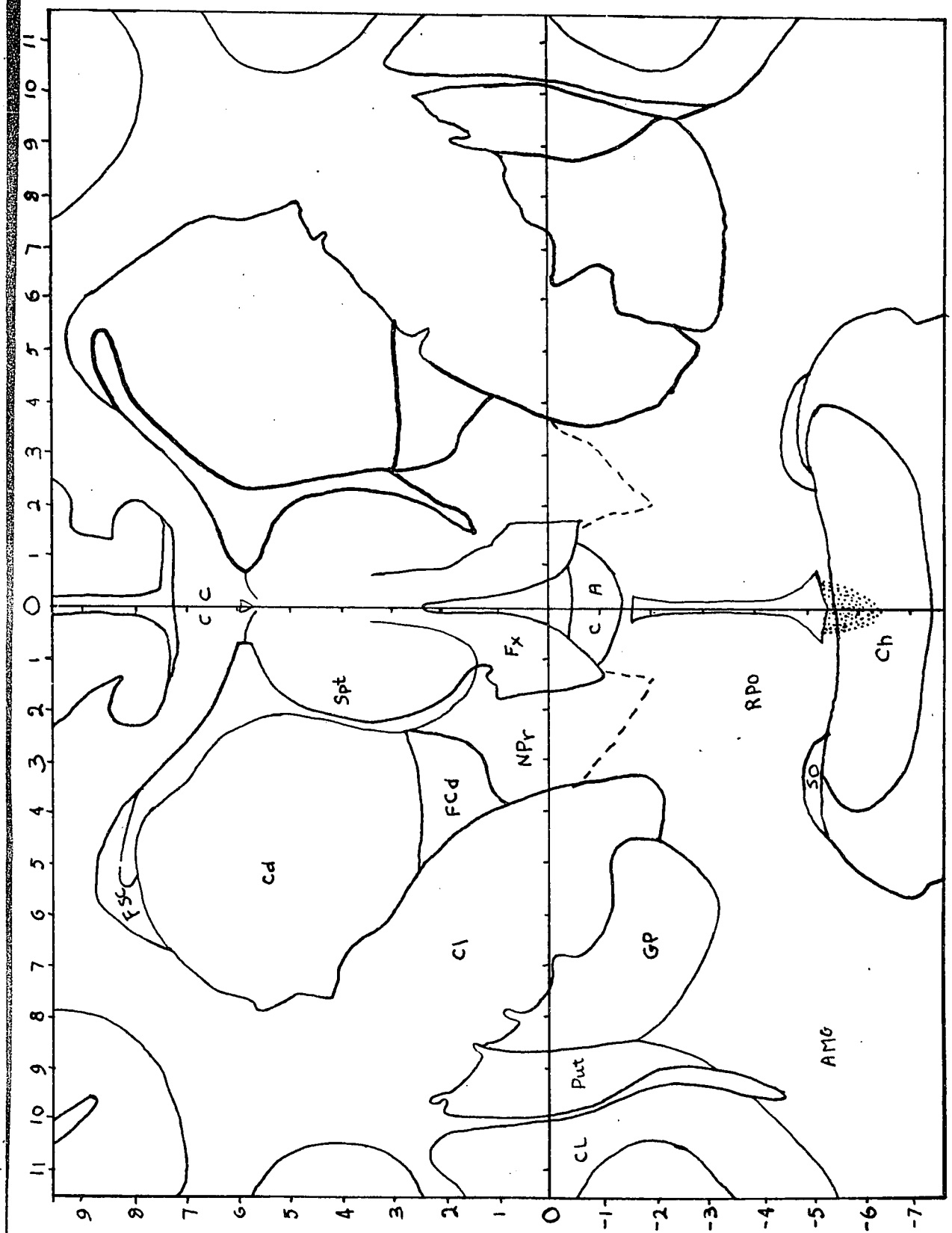

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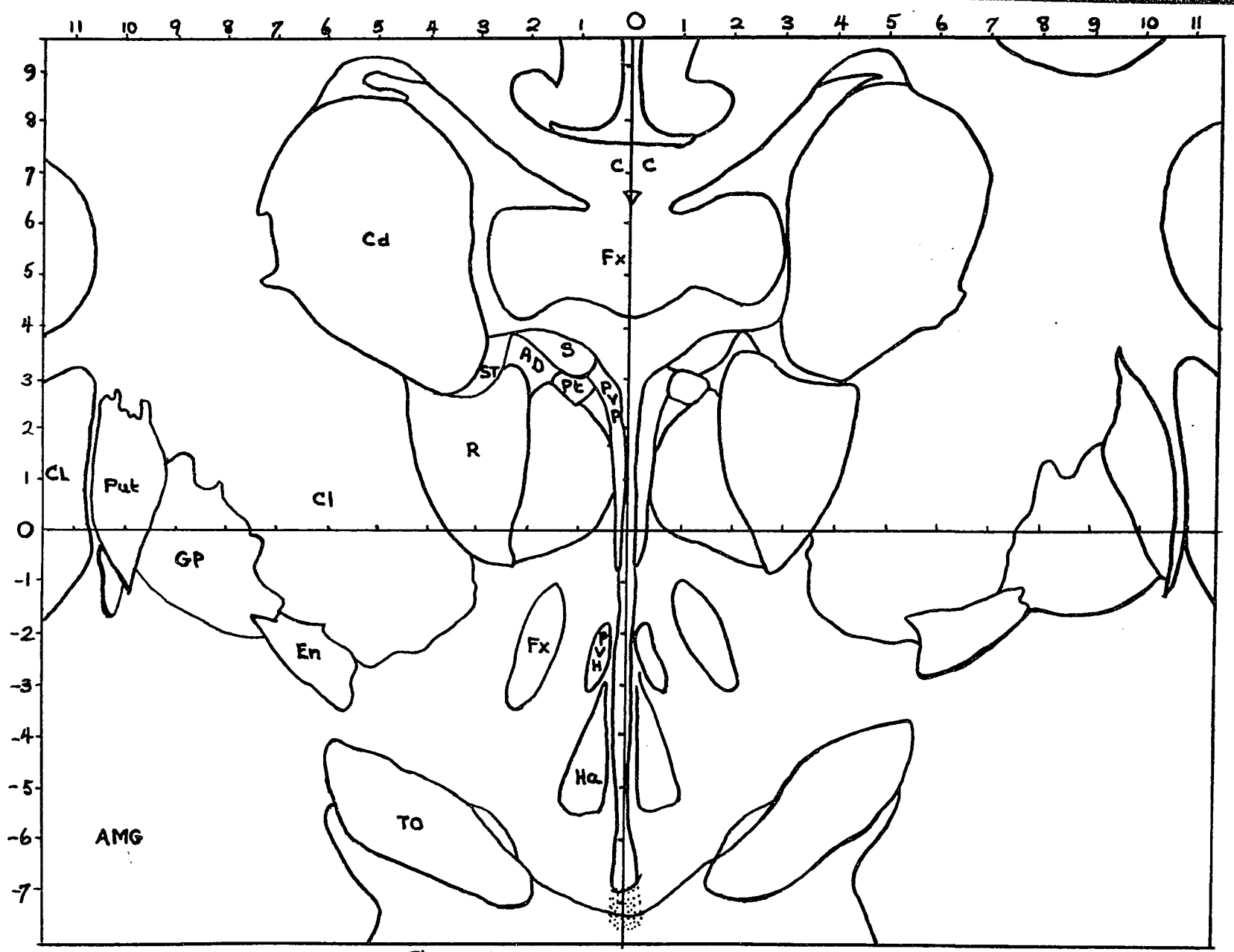

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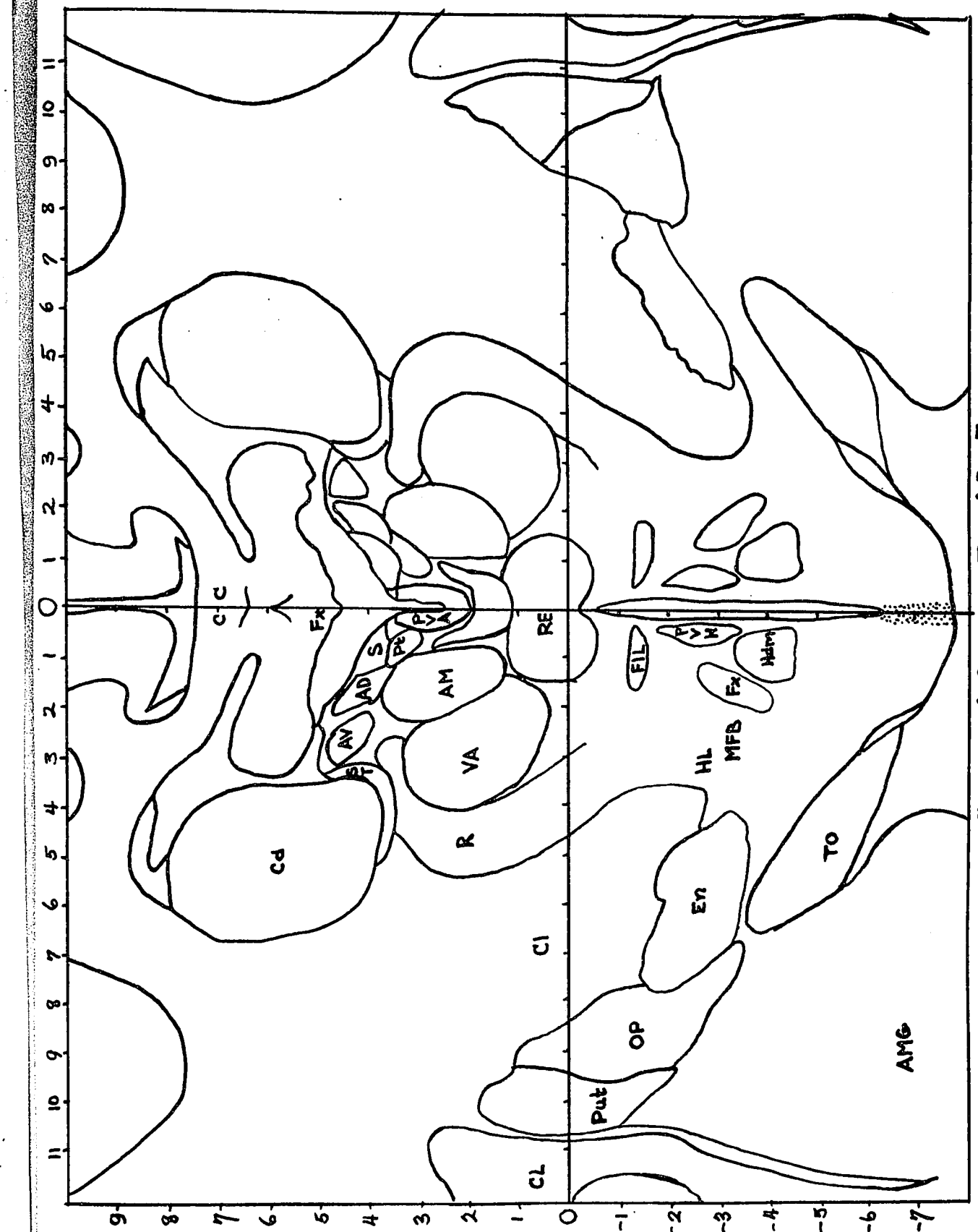


Sec. 140 Fr. 12.5

- Histological examination.

Degrees of various morphological kinds of retraction bulbs are found scattered within this area. These bulbs are round or oval with tail-like processes which are deeply stained with colloidal silver. These peculiar degenerative structures are most abundantly found on either side of lesion. At region of ventral hypothalamic decussation, that is at supraoptic and infundibular region, the retraction bulbs decrease proportionately away from the lesion.

Associated with these various transitional morphological degenerative changes are to be found the true terminals in the form of intense darkly stained rings of various sizes but of unique and constant morphology. The terminal boutons are present within and around the supraoptic and suprachiasmatic nuclei and to a lesser degree within the ventro-medial hypothalamic nucleus. The infundibular region is also permeated by boutons.

Observations (Cont'd.):

Of thalamic nuclei, boutons in the form of rings are seen within the medial geniculate body of both sides as well as within the pulvinar also of both sides.

Obaervations:

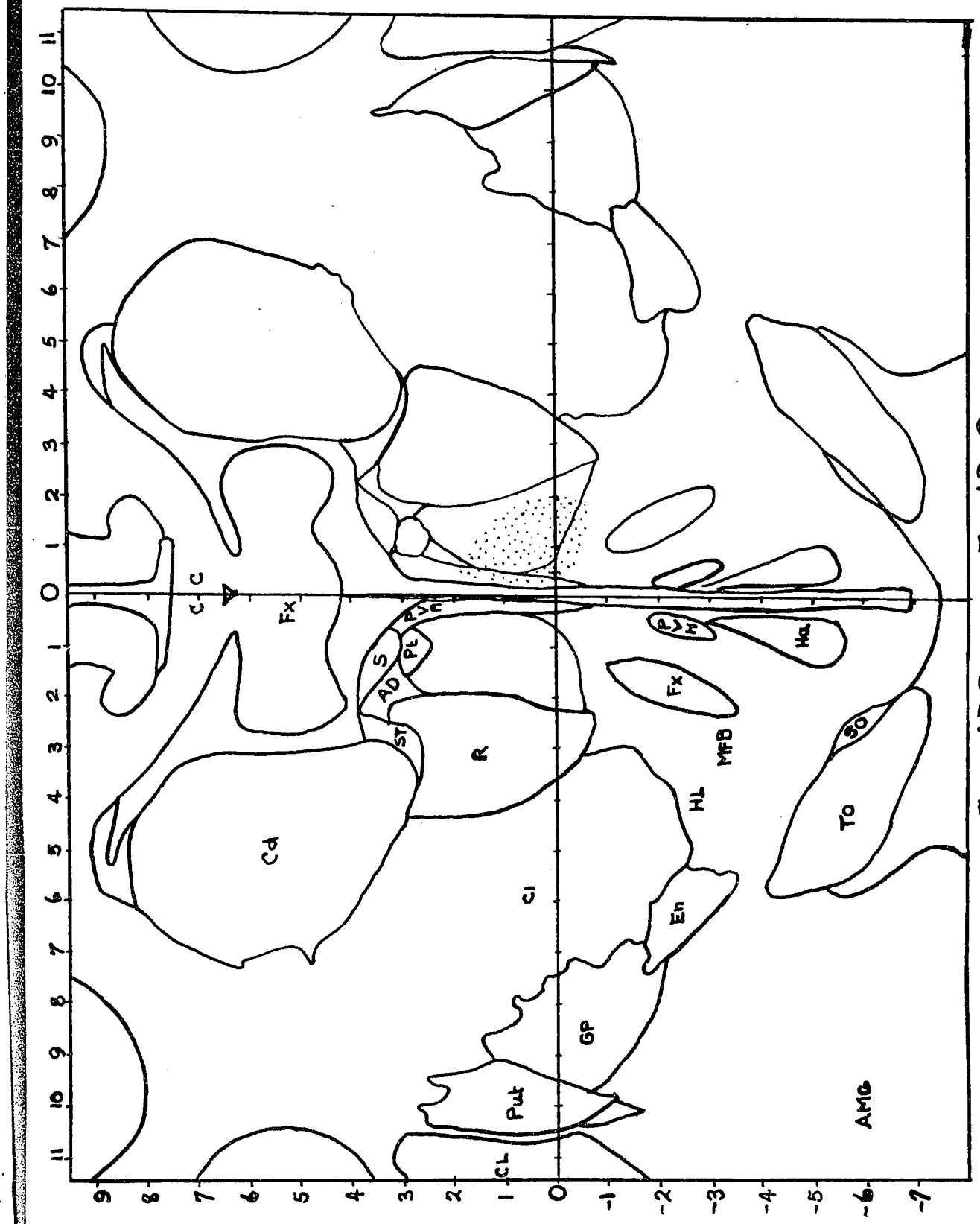
Cat 26AB - Post-operative period of 4 days.

- Perfused with 100 to 200 cc. of cold acetone and fixed in above solution for 48 hours in cold with one change after the first 24 hours.
- Insertion of electrodes according to Horsley-Clarke stereotaxic coordinates:-

Frontal	12
Lateral	1
Horizontal	-1

- Localization of lesion.

Partial destruction of following nuclei:
n. periventricularis anterior, n. commissuralis inter-antero medialis, n. reuniens, n. anterior medialis, n. centralis medialis, n. rhomboidalis, most medial part of n. ventralis medialis, and dorsal hypothalamic area.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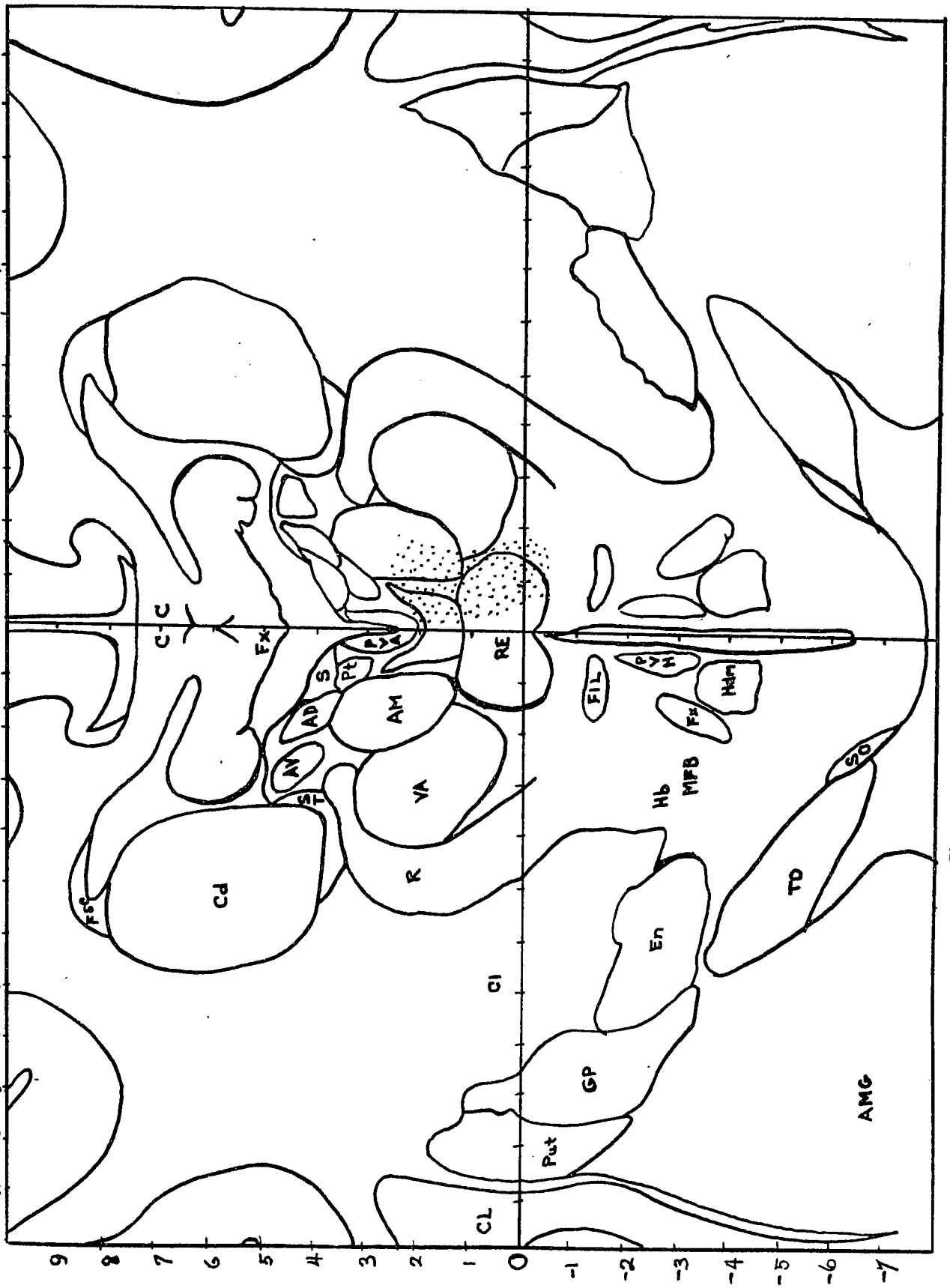

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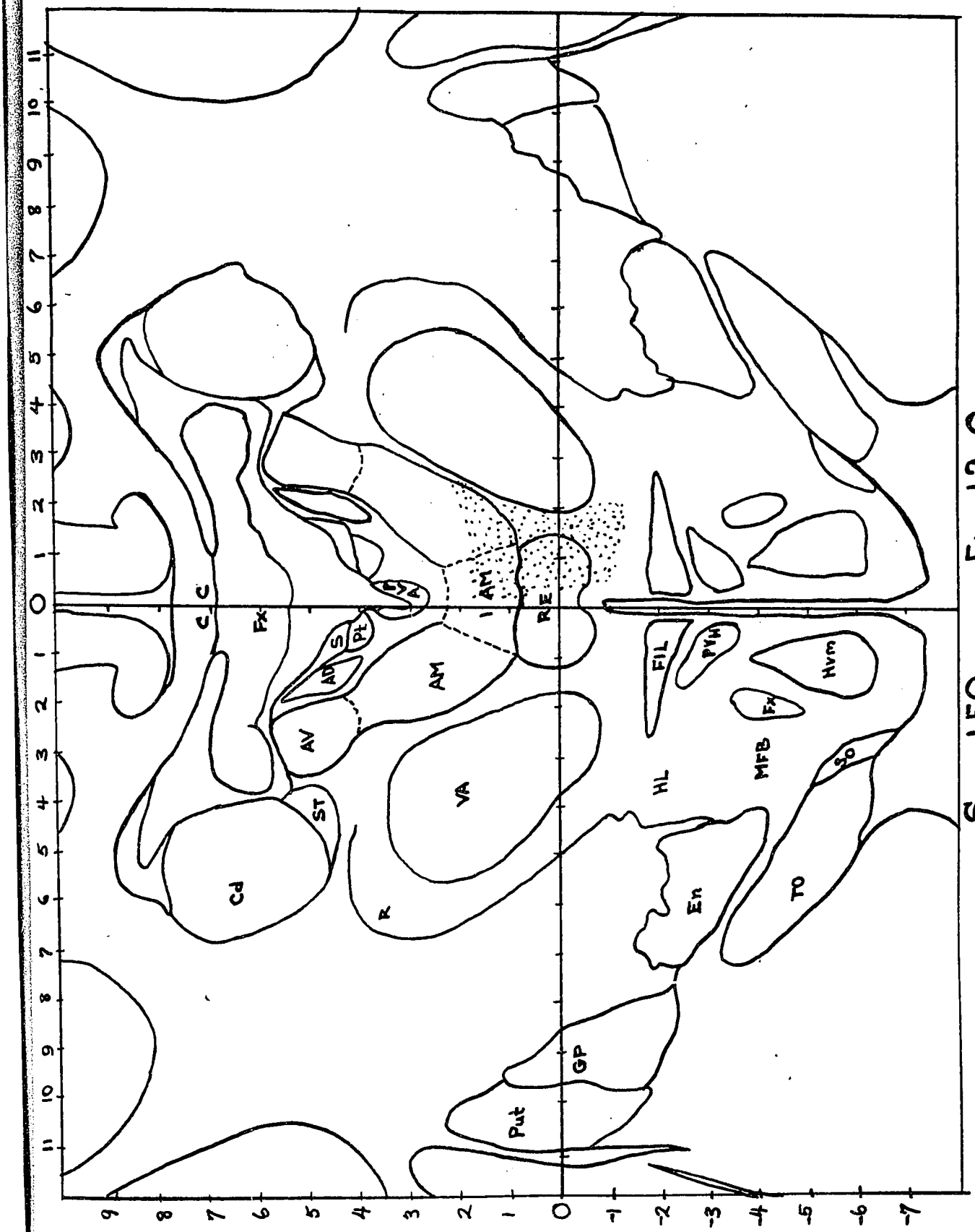
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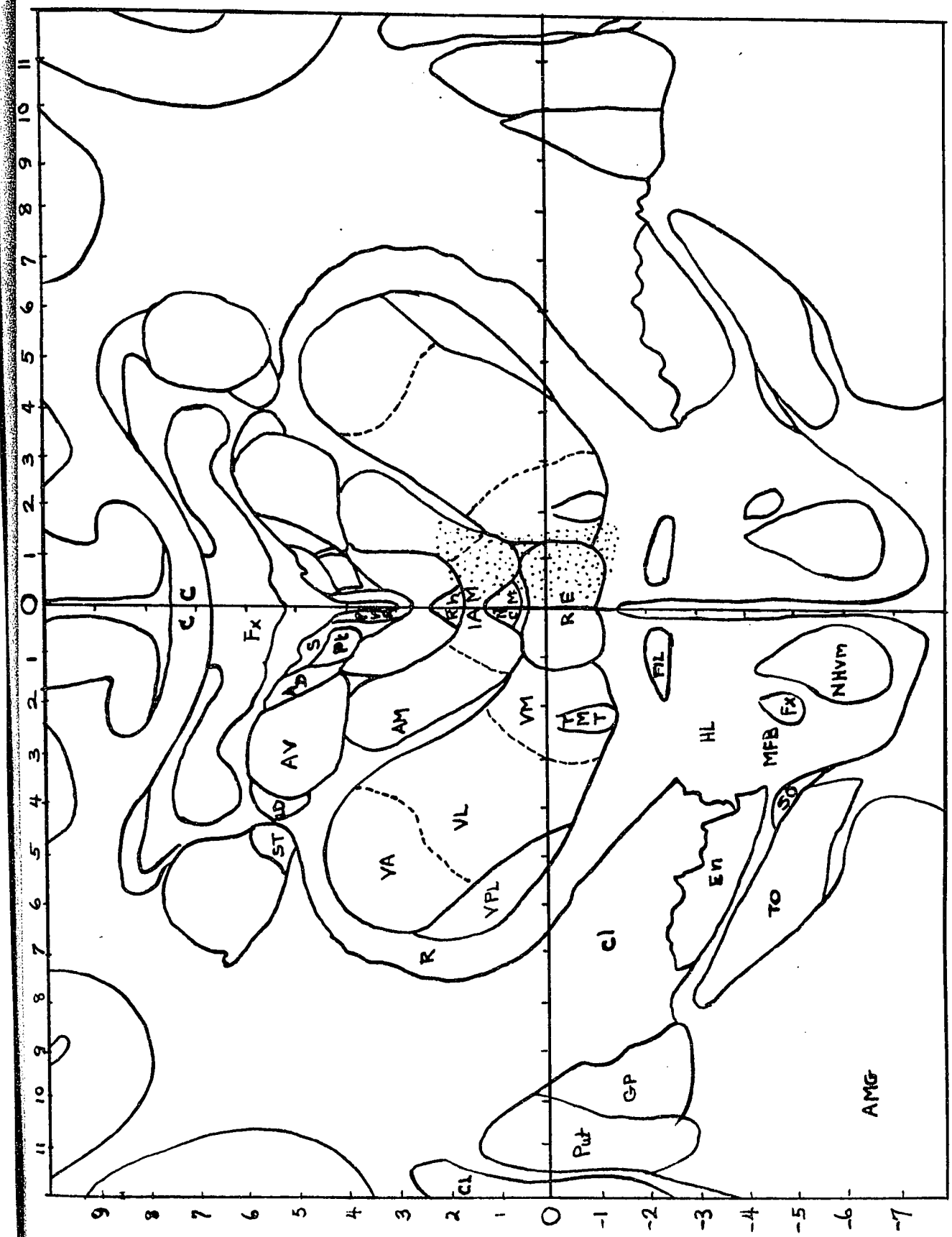

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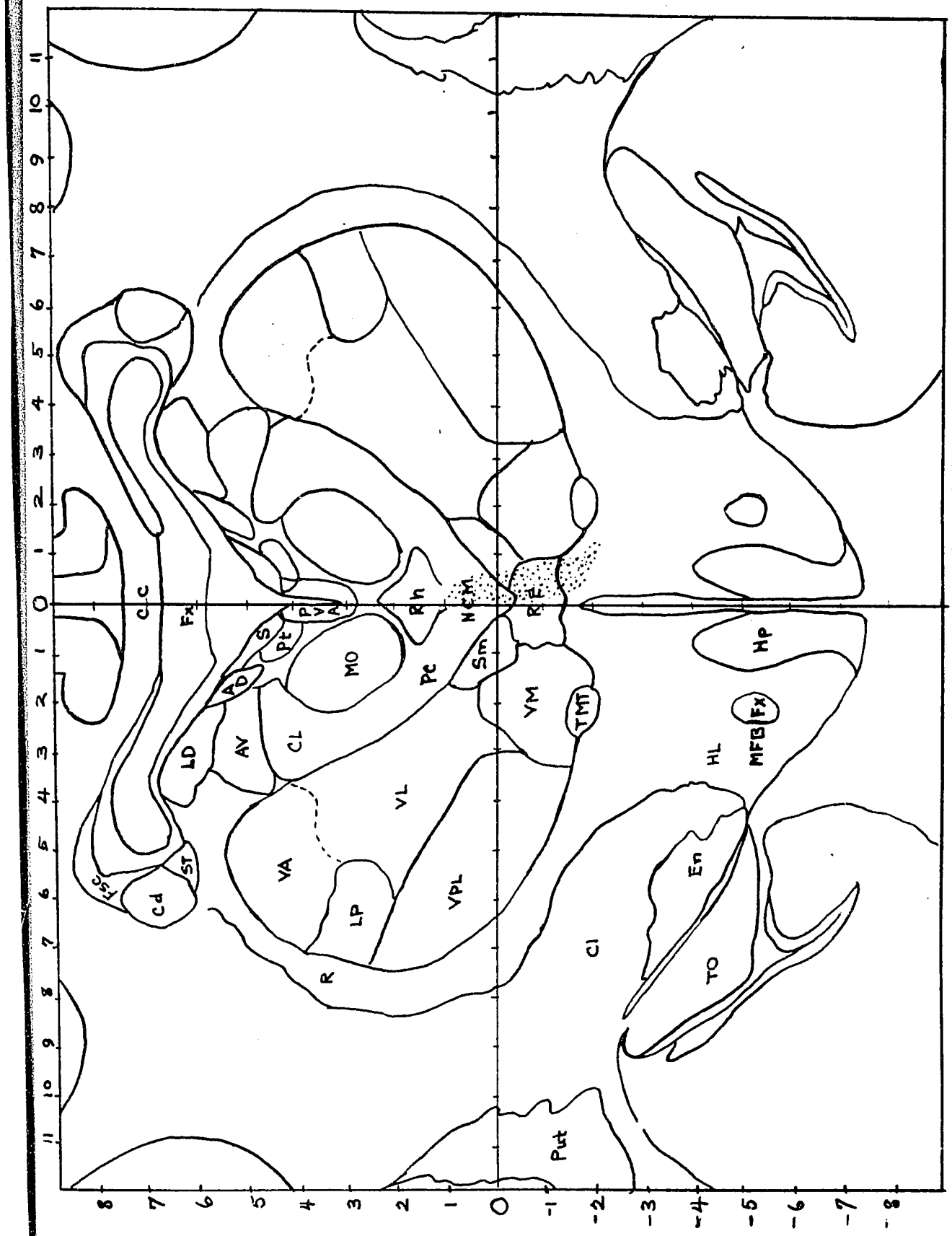
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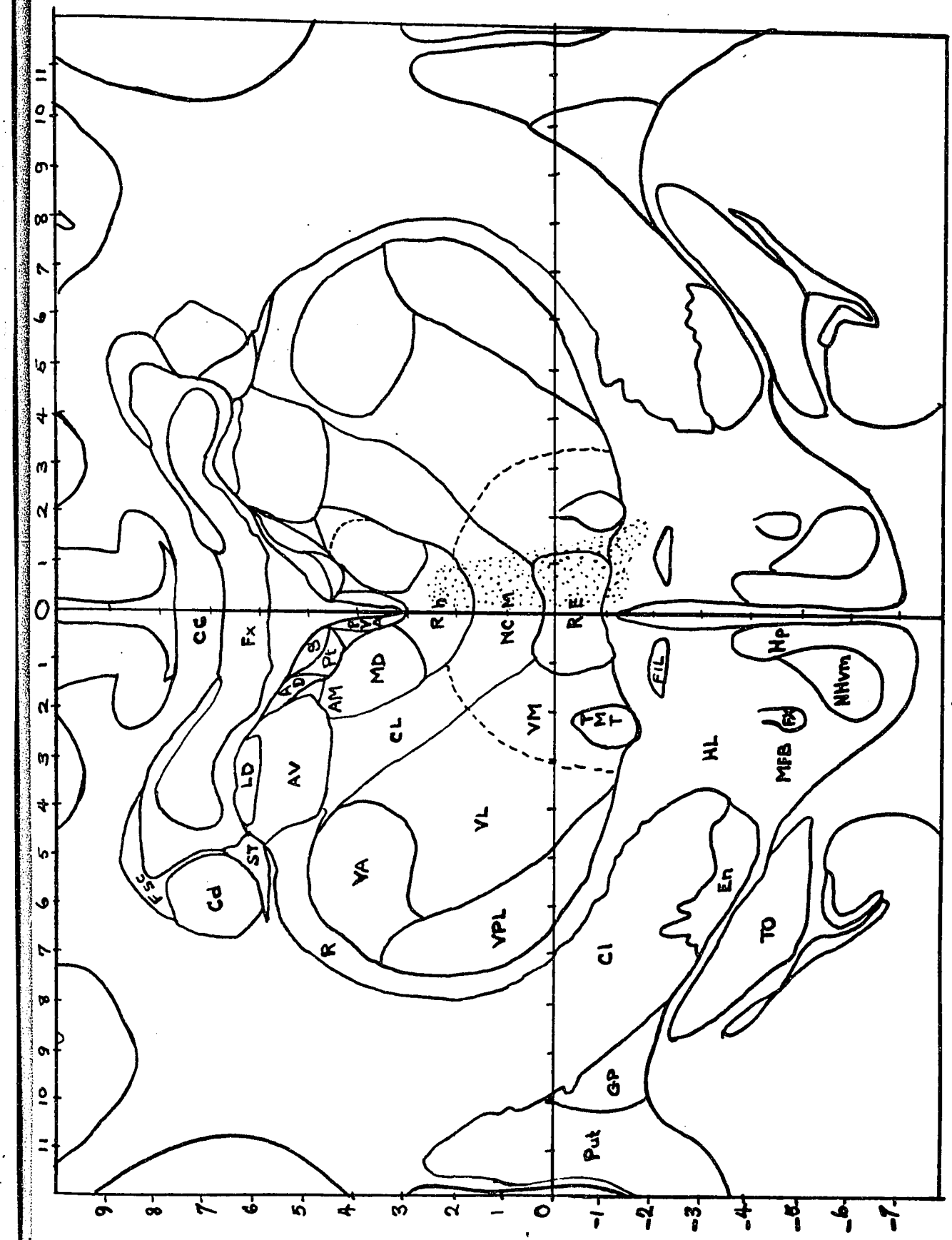
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- Histological examination.

A few scattered terminals are seen within the periventricular hypothalamic area. Coarse, heavily myelinated and degenerating fibers are seen travelling with the normal fibers of the paraventricular-hypophysial tract.

Ring-like terminals are found within the medial and lateral septal nuclei.

Observations:

Cat 27A - Post-operative period of 4 days.

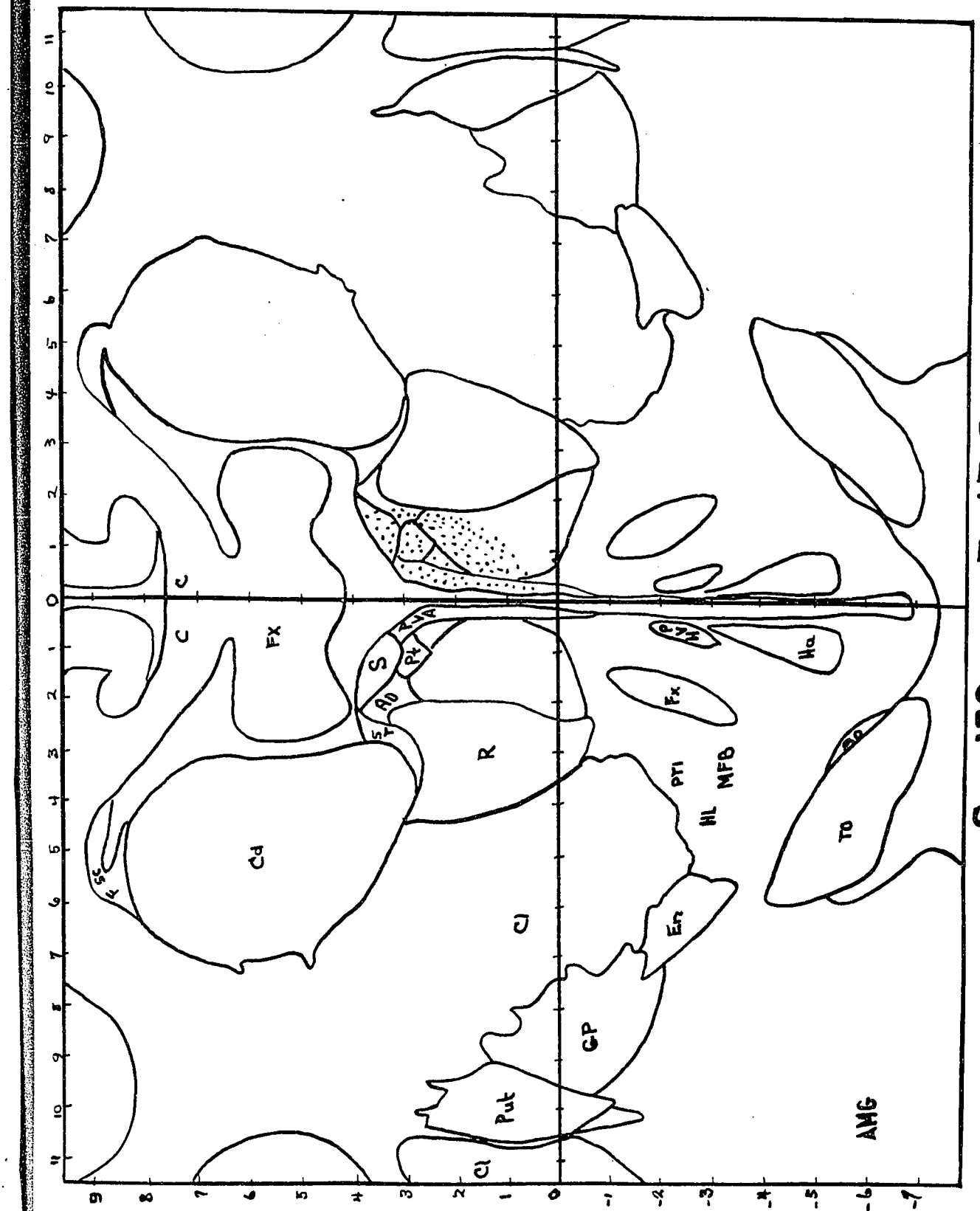
- Perfused with 100 to 200 cc. of cold acetone and fixed in above solution for 48 hours in cold with one change after the first 24 hours.
- Insertion of electrodes according to Horsley-Clarke stereotaxic coordinates:-

Frontal	10.0
Lateral	1
Horizontal	+3

- Localization of lesion:-

Total destruction of the following nuclei: n. periventricularis anterior, n. anterior medialis, n. anterior dorsalis, n. commissuralis inter-anteromedialis, n. reuniens, n. rhomboidalis, n. centralis medialis, n. submedius, n. medialis dorsalis, n. habenularis medialis and lateralis, n. centrum medianum, and in part, the most postero-medial aspect of n. lateralis dorsalis and ventralis medialis.

Those nuclei that border the mid-line area were in part affected on opposite side of lesion due to irradiation.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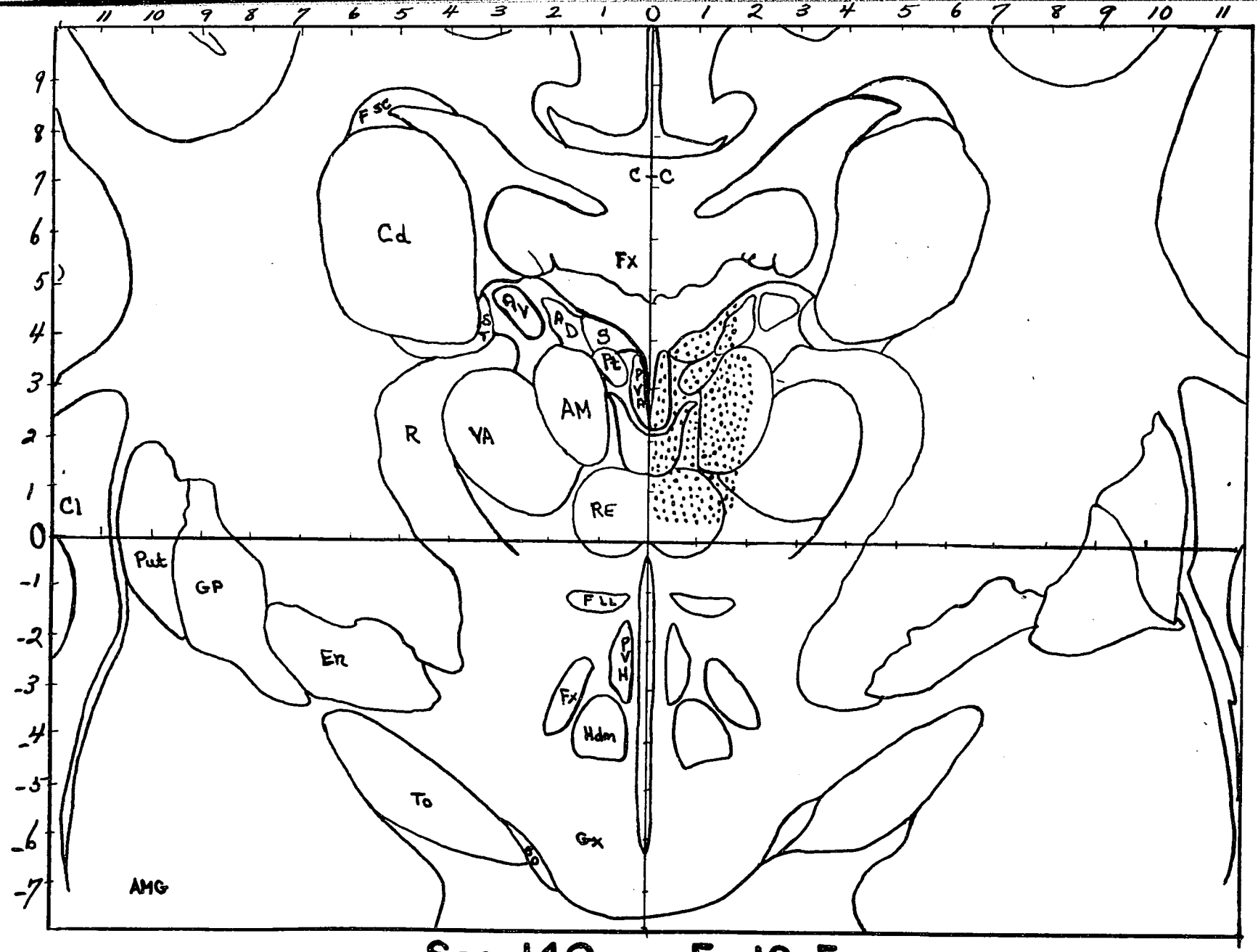

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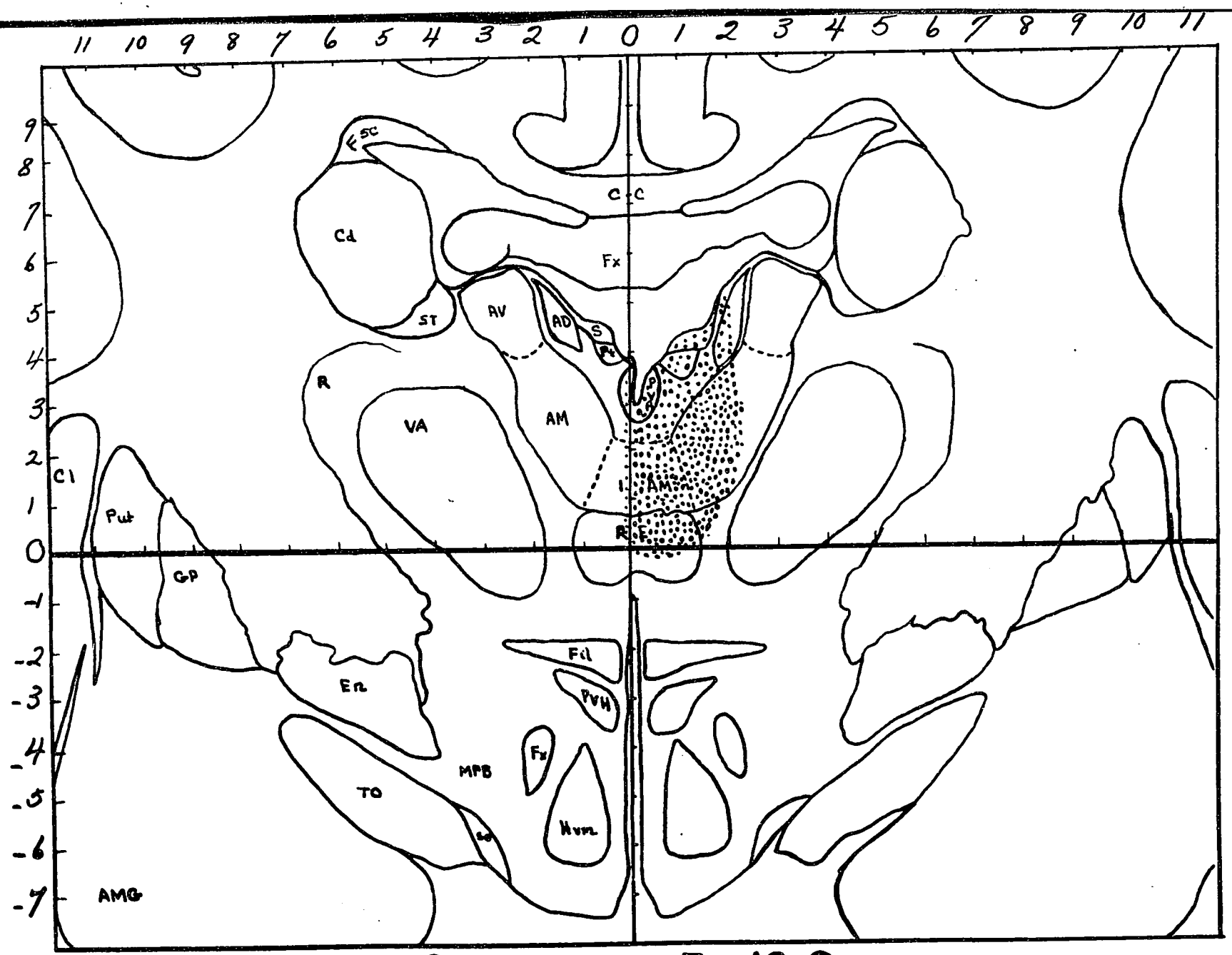

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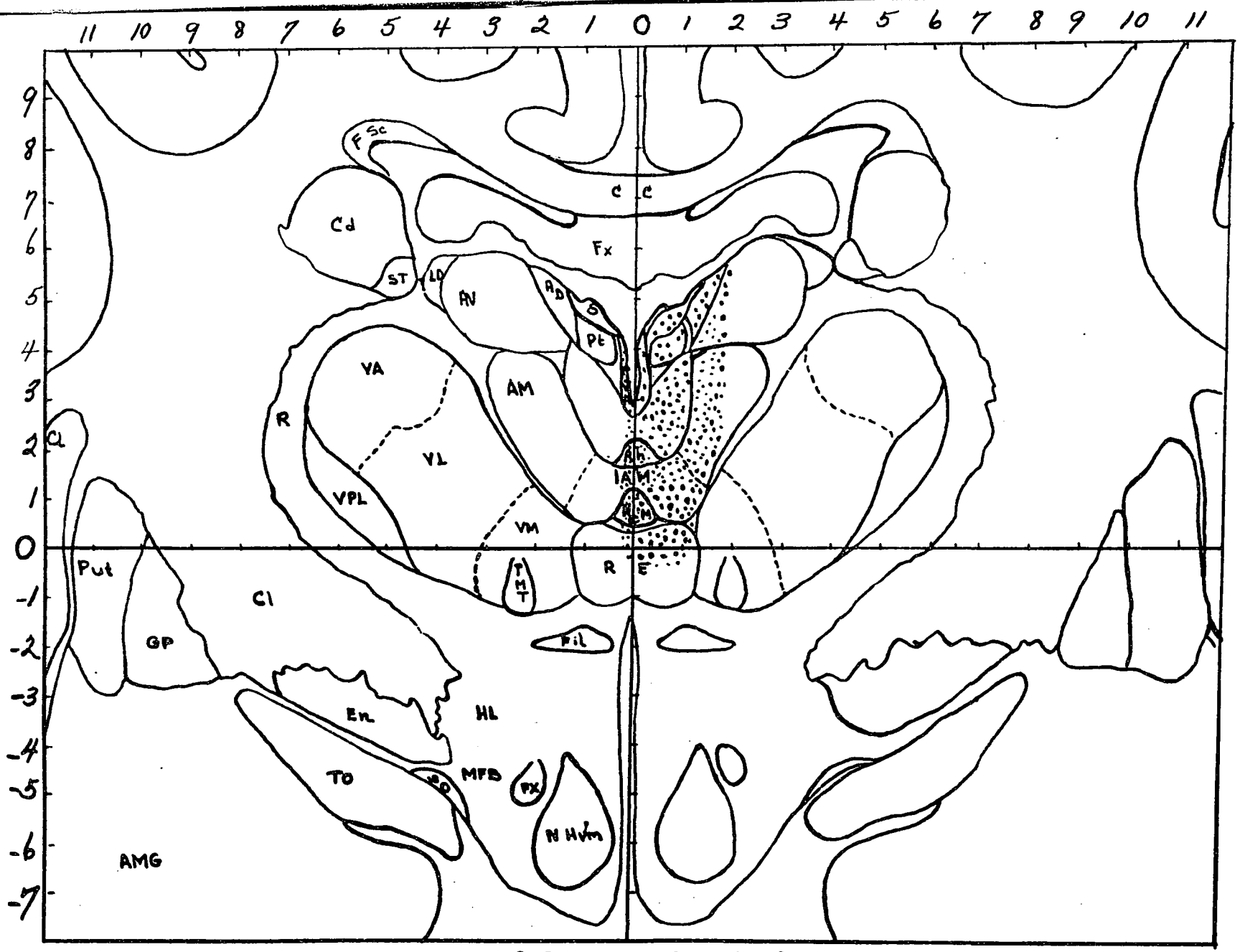

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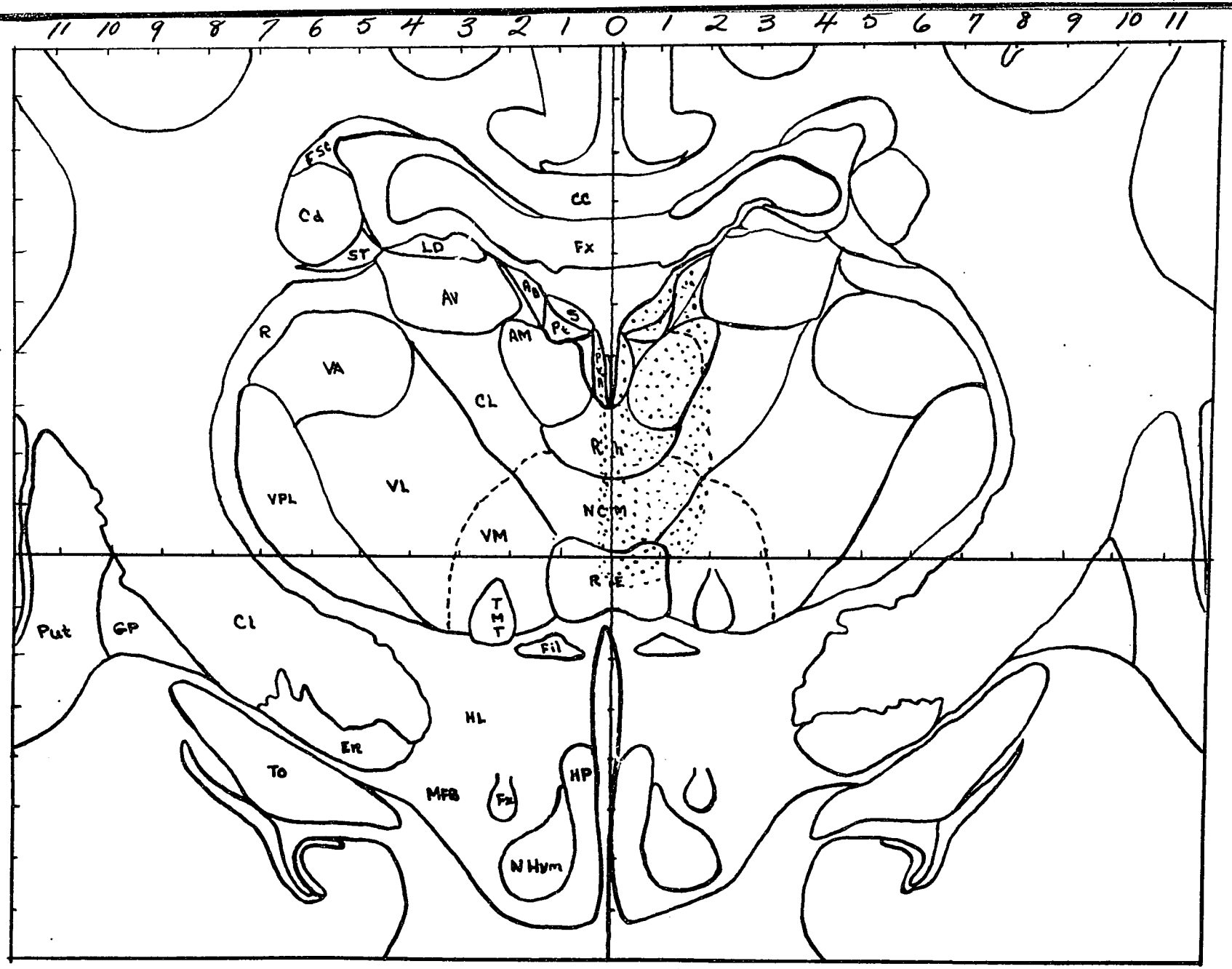

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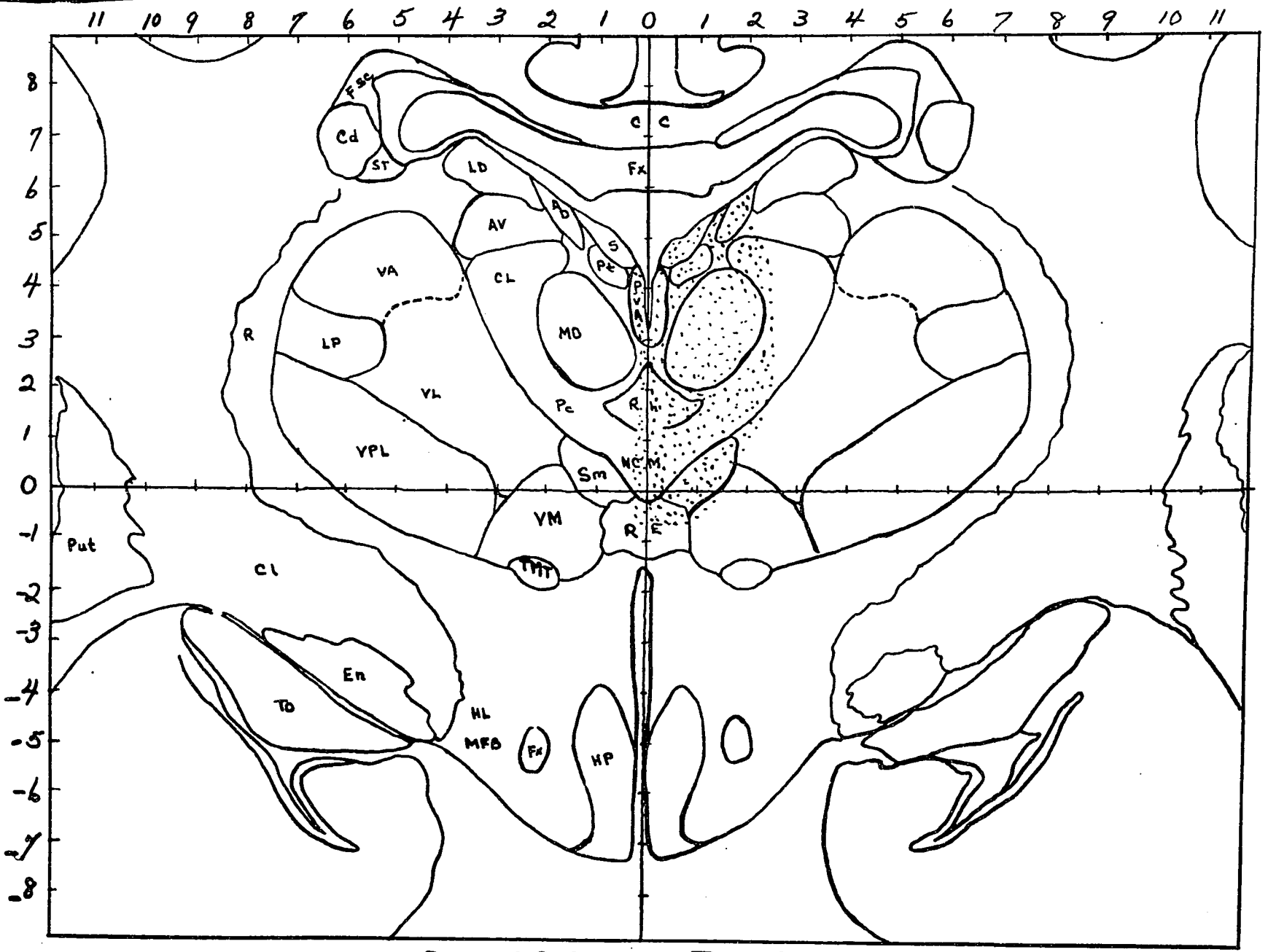

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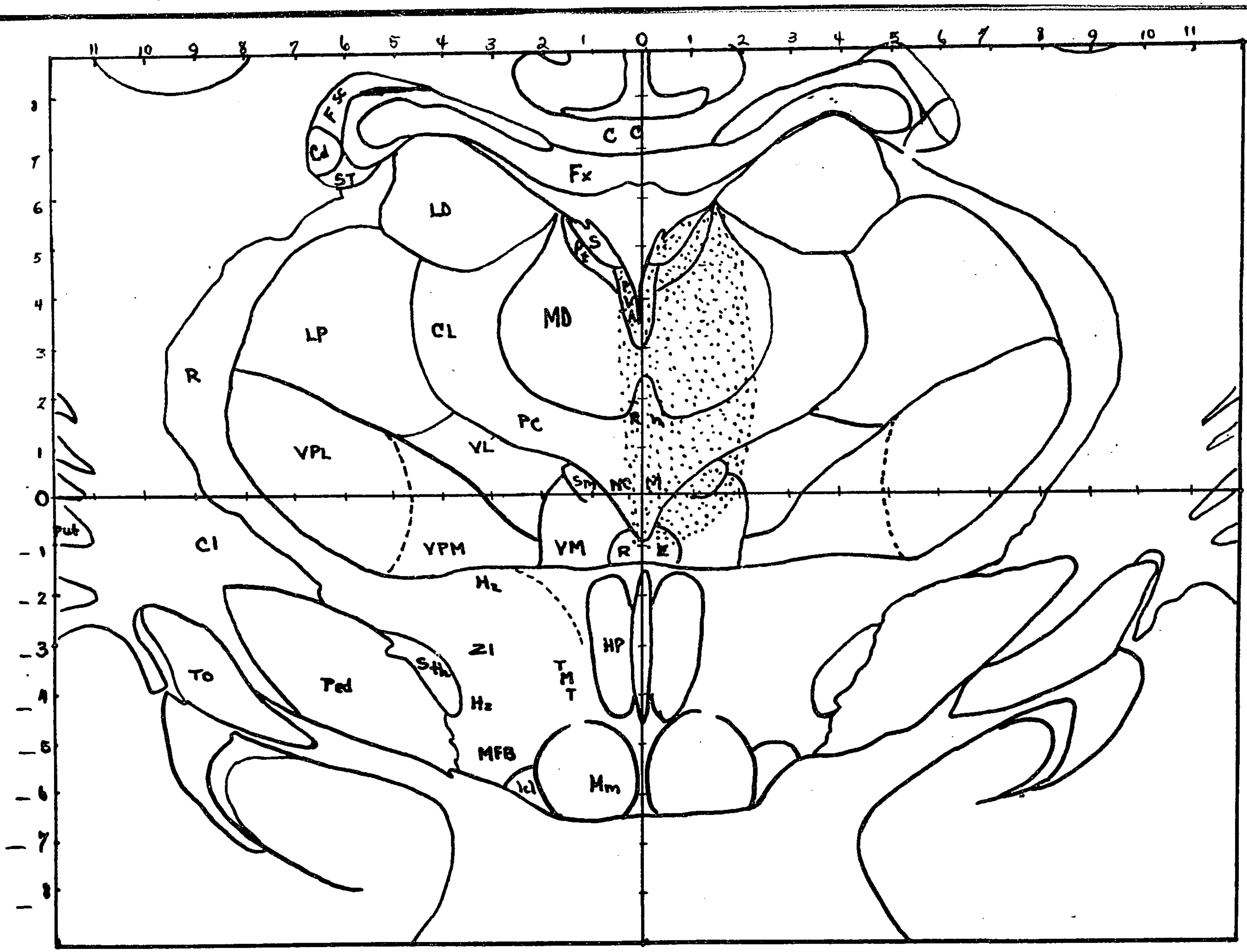
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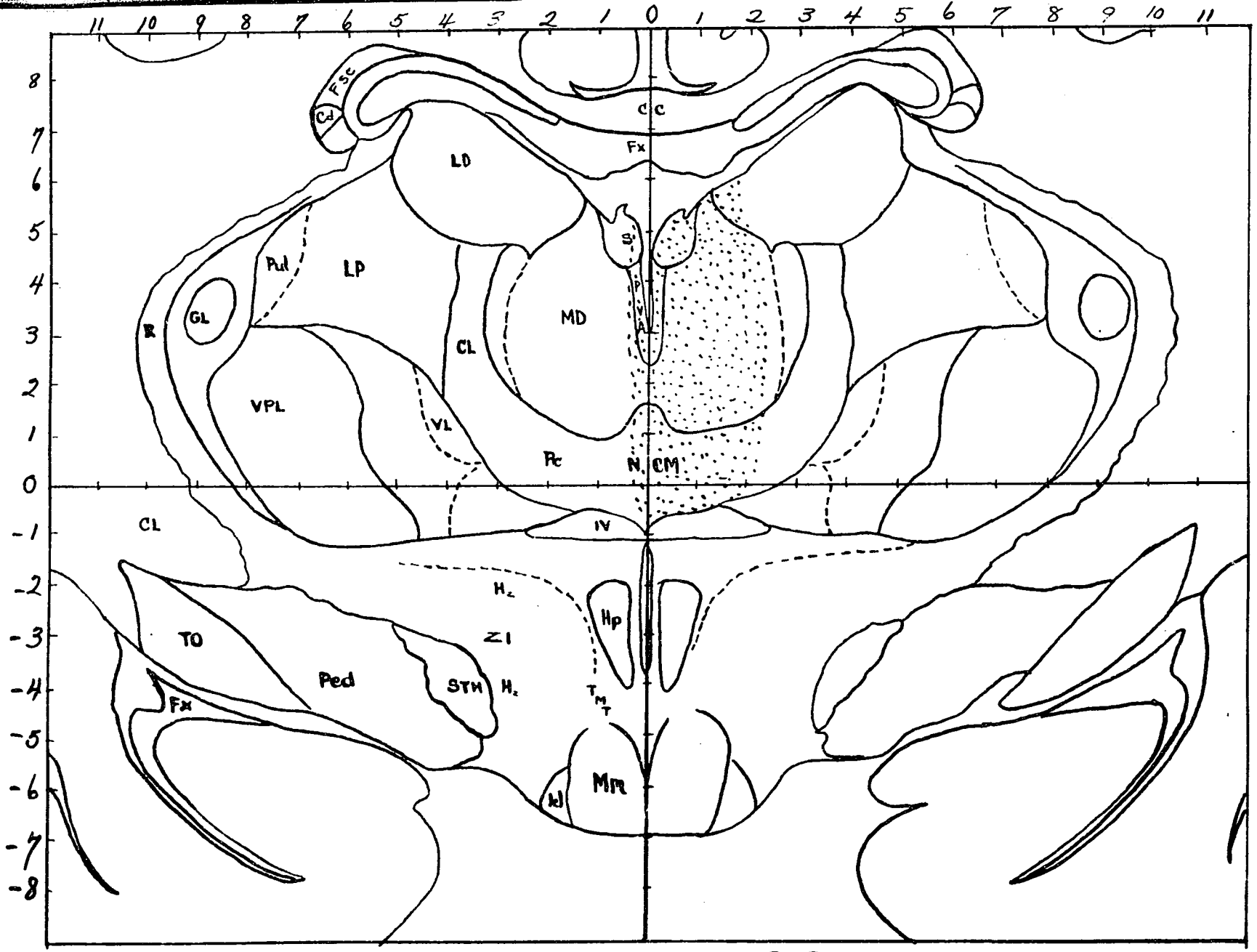
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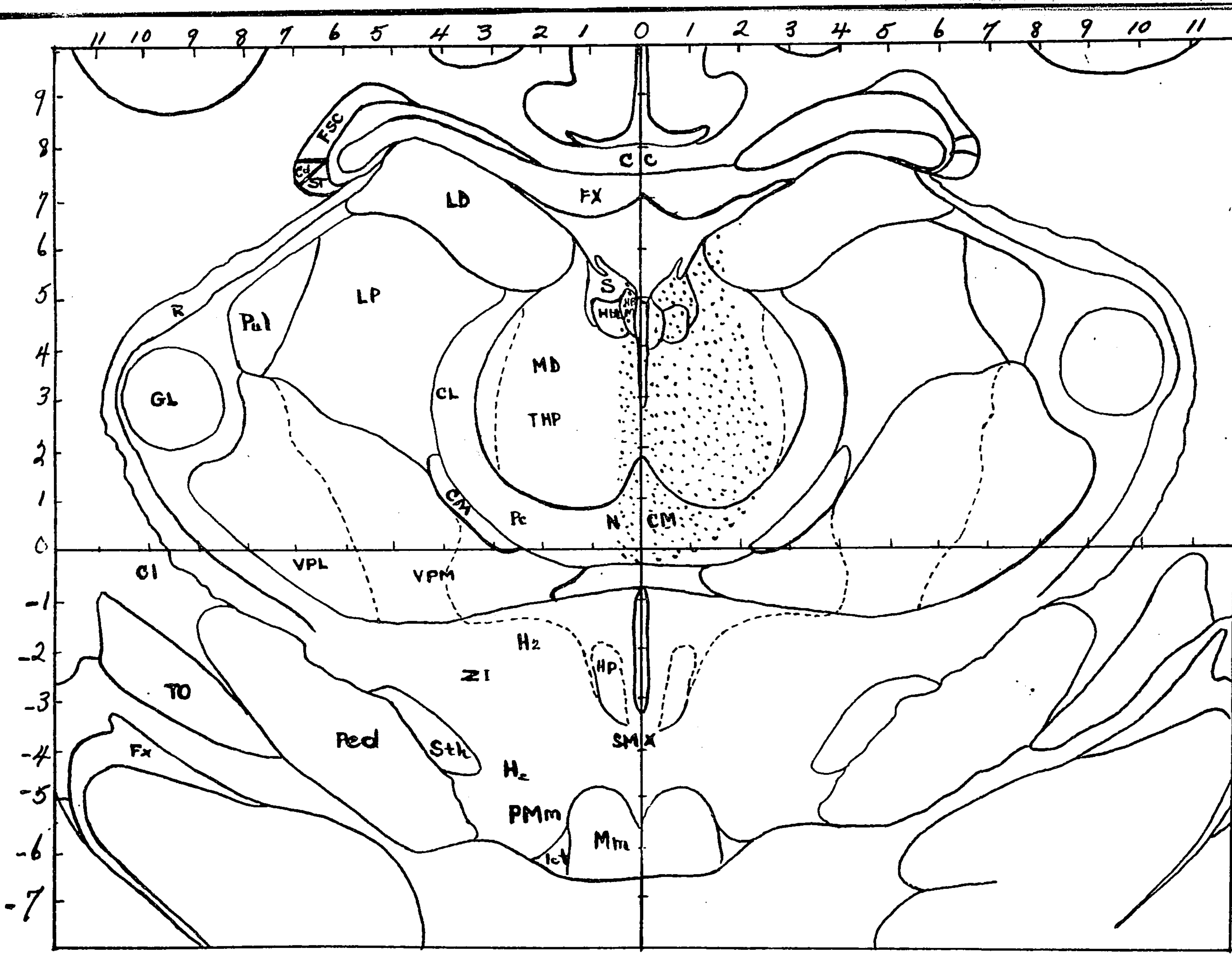
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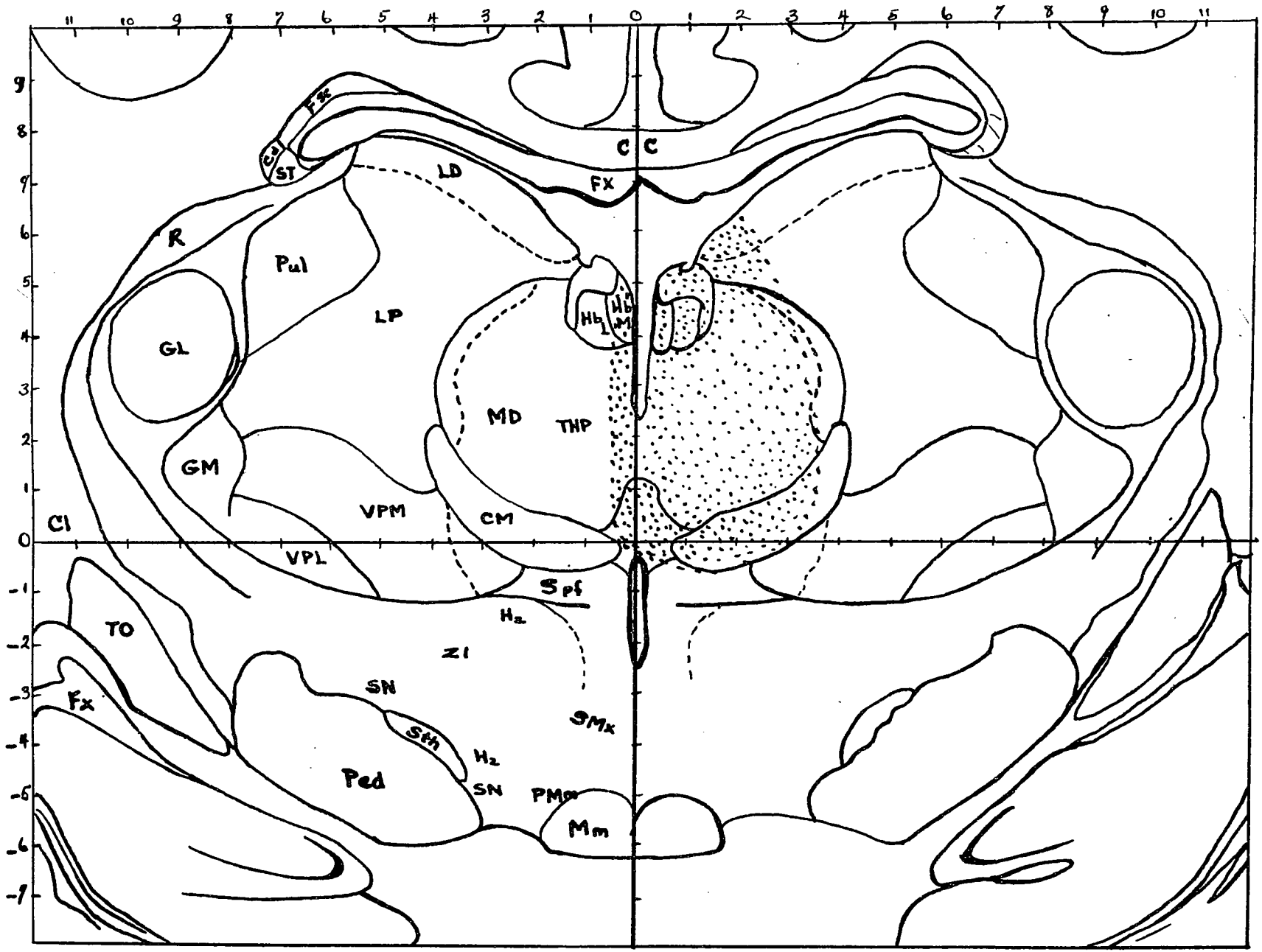
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- Histological examination.

Severe advanced terminal degeneration of paraventricular nucleus on same side as lesion with slight degeneration (boutons) of opposite side of lesion.

Coarse and heavily myelinated degenerating fibers are seen coursing with the normal unmyelinated fibers of the paraventricular-hypophysial tract.

Observations (Cont'd.):

Terminal reaction, as in cat 26AB, is also seen in medial hypothalamic area (medial periventricular nucleus).

The medial and lateral septal nuclei show degrees of advance degeneration with terminal rings scattered among the neurites.

Inconclusive degenerative indications are present within the nucleus ventralis postero lateralis.

CHAPTER IV

DISCUSSION

1. The Technique.— In view of the observations, it is advisable to re-evaluate some of the loosely designated tracts (origin, course, termination and direction of conduction) which have been based primarily on normal material and to a minor degree on experimental data.

Since Cajal, experimental neuro-anatomists have gradually deviated from the chartered course set about by him, i.e. the working out of the finer details of the nervous system by silver techniques, and have fallen back to techniques which, though useful for many purposes e.g. the Marchi and Nissl stain, yet inefficient in giving details about certain areas of the brain, especially the hypothalamus which, aside from the large, well-defined myelinated tracts, shows hardly any myelination.

The Nissl retrograde method is based on the appearance of the Nissl granules at various stages of disintegration. This method, though excellent for many purposes, introduces errors which are hard to cope with since they are misleading in interpretation (nuclear eccentricity and peripheral concentration of Nissl granulars). Furthermore, lack of agreement is based on the animal used, the post-operative time and, most emphatically, as it has been recently proven, on the age of the animal at the time of operation (Brodal, 1940). Though to a limited extent, yet detrimental to research, another criterion indicative of degeneration has crept within the research laboratories. This criterion is based on the

proliferation and accumulation of glia cells along degenerating nerve pathways. When applied to degenerating, well-circumscribed and heavily myelinated tracts, the method is commendable.

By using the number of glia nuclei per unit area, the above method has been used extensively in the study of diffused tracts which often show light or no myelination whatever. This method is even more doubtful when one considers that haematoxylin and eosin are employed.

The importance of the fixatives has also been underestimated. For instance, different pictures of the nerve cell content are obtained if the tissue is fixed in any one of the mentioned fixatives: formalin, formol-alcohol, chloral hydrate, acetone and Zenker acetic fixed tissue. If the paraventricular nucleus is stained with Nissl reagent (toluidine blue or cresyl violet) the cell body and content stand out most elaborately: centrally located nucleus, surrounded by a perinuclear clear space and with Nissl bodies densely aggregated in various morphological forms at periphery of cell body.

With chloral hydrate as a fixative, the nucleus and Nissl granulars appear in various morphological forms depending on the time factor of tissue fixation. In most instances, the Nissl granulars appear in powder-like form and the nucleus shows up as a clear space.

With acetone, and above all, Zenker acetic, the cell body and its constituent delicate elements are preserved with the greatest care and always appear the same no matter how long the fixative has acted on the tissue.

Bodian recognized these factors and therefore conceived the method of staining neurofibrils with colloidal silver by using protargol

as the active reagent and the Bodian fixative at all instances as the passive factor. The disadvantage of the above mentioned technique lies on non-technical grounds and has been thoroughly discussed in Chapter II.

Of the innumerable methods worked out, all based on the reduction properties of colloidal silver, for bulk, frozen or serial paraffin sections, none fulfil the conditions which were planned before using the technique for experimental purposes.

The procedure of our technique has no subjective factors worth considering. The chemicals are easily prepared and can be stored over a period of time. The time factors within each solution have been standardized and therefore the results are always constant. Furthermore, it is applicable to serial paraffin sections and therefore one is able to stain alternate sections with various other stains: Nissl, Heidenhein, etc. The method stains equally well the normal or the various stages of degenerated fibers whether it be in cortex, interbrain, stem or cord. The greatest advantage lies in its ability to stain terminals, either they be rings, bulbs, or free endings. These latter morphological synaptic endings are stained more intensely than the normal fibrils which are black against a light background.

Other advantages of the stain are worth mentioning for it adds details which cannot be gleaned from Marchi or Nissl type of stains. First of all, being a fibrillar stain, it stains equally well myelinated and unmyelinated degenerating fibers. These degenerating fibers are seen on a background of normal fibers; thus one is able to determine rather precisely which fiber groups are affected by the lesion, and which remain normal.

This makes the analysis of these preparations much more time-consuming than that of Marchi preparations. It allows one to note the dropping out of fibers since all fibers are stained and it stains both axons and dentrites. However, studying silver preparations is time-consuming but one is repaid by the added information obtained.

2. The Experiments.— In Cat 1AZ, where the medial and lateral septal nuclei have been completely destroyed (diagonal band of Broca only in part), terminal reactions in the paraventricular nucleus and beading of a few fibers within the paraventricular-hypophysial tract were produced.

The afferent fibers to the paraventricular nucleus arise either from septal nuclei or from the cortex. These fibers travel, in part, with the medial forebrain bundle through the lateral hypothalamic area and with the periventricular system through the medial preoptic area and medial hypothalamic area as the septal or cortical component of the periventricular fasciculus. The beaded fibers found along the bundle of the hypothalamic-hypophysial tract (which partly arises from the paraventricular nucleus) cannot be explained on the basis of these facts since transneuronal degeneration has not been reported as a common phenomenon (except for the optic fibers). It is possible that these beaded fibers have a ventral hypothalamic distribution. A septal distribution to the ventromedial and dorsomedial hypothalamic nucleus has been reported in the literature. Terminals have not been found within the pars nervosa of the pituitary nor within any other hypothalamic nuclear mass.

Cortical ablation experiments of the frontal lobe now being carried out by Auer have shown that a frontal component (passing through the septum)

does reach the paraventricular nucleus as well as various nuclear groups of the infundibular area. From these experiments, it may be suggested that the beaded and swollen fibers seen coursing with the fibers of the paraventricular-hypophysial tract are the cortico-hypothalamic fibers destined for the infundibular region to be distributed to the ventromedial and possibly to the dorsomedial hypothalamic nucleus.

Besides the terminal fibrillar reaction seen within the paraventricular nucleus, it is observed that cytological changes are present within the main magnocellular portion of the paraventricular nucleus (toluidine blue preparations). Pyknotic changes of the nucleus and degrees of granulation of the Nissl material is apparent.

This evidence may lead one to conclude that the neurites of the paraventricular nucleus are undergoing retrograde changes due to severence of its axons at the region of the septum. This would imply that the paraventricular nucleus has a septal or cortical representation besides receiving corticofugal fibers.

It has long been known that the paraventricular nucleus sends a component to the pars nervosa of pituitary via the hypothalamo-hypophysial tract. It has also been observed by various authors that in lesions of the hypothalamo-hypophysial tract, the retrograde changes within the paraventricular nucleus do not involve all of the magnocellular elements. The major portion of the nucleus shows no changes whatever.

The possibility exists for corticopetal fibers arising from the magnocellular portion of the paraventricular nucleus which course through the septum.

The above observation should be accepted cautiously for retro-

grade changes are difficult to interpret, especially when dealing with cells in which the Nissl granules are disposed at periphery.

The lesion in Cat 2A has destroyed the caudate nucleus, the putamen, the globus pollidus, part of the amygdaloid complex, the olfactory cortex and the anterior limb of the internal capeule.

The hypothalamus is extensively involved; the medial, lateral and dorsal hypothalamic area, paraventricular, supraoptic, suprachiasmatic and ventromedial hypothalamic nucleus, and infundibular area show degeneration.

These efferents to the hypothalamus course as capsular fibers or as components of the medial forebrain bundle. The capsular fibers arise from the cortex, which is apparent from the terminal degeneration within the midline nuclei and dorsomedial thalamic nucleus and in part from the lentiform nucleus. The possibility of a lentiform projection to the midline nuclei cannot be entirely ignored.

The evidence suggests that the medial forebrain bundle has the heaviest origin from the ventromedial base of the hemisphere rather than the hypothalamic nuclei as has been suggested by various authors. It is also observed in the cat, best in horizontal sections, that the medial forebrain bundle does not increase in size as it courses posteriorly towards the stem. This fact would further suggest that the bundle has its main source of fibers from anteriorly placed areas and that hypothalamic nuclei hardly contribute to the bundle. The bundle would primarily be efferent to hypothalamic centers. In part, the projection to the paraventricular nucleus is the cortico-(septo)-hypothalamic component coursing with the medial forebrain bundle which has been destroyed

by the lesion.

A strio-hypothalamic component within the bundle to the above mentioned hypothalamic nuclei is conceivable though further evidence is necessary before an appropriate evaluation can be made.

It is interesting to observe that a tract reaches the paraventricular nucleus (see photographs) from the direction of internal medullary lamina and reticular nucleus of thalamus, which tract may well be, among others, of striatal origin.

Gurdjian assigned a heavy projection of cortico-hypothalamic fibers travelling with the medial forebrain bundle to the mammillary nuclei. Cat 2A has received extensive destruction of the bundle and no terminals have been found within or around the mammillary complex.

Crossing cortico-hypothalamic fibers which decussate with the supramammillary bundle, are clearly seen in horizontal sections, but no terminals have been found at their vicinity. Evidence is present which indicates projections of crossed cortico-thalamic fibers to mid-line nuclei (n. centralis pars lateralis), anterior thalamic nuclei, and to the habenular complex which receives the stria medullary component of the stria terminalis.

Cat 16A has received less destruction, with lack of injury to the olfactory cortex and related structures. The lesion has been localized to the head of caudate and to the anterior limb of internal capsule.

As stated, transneuronal degeneration is rare, and since most of the caudate fibers are relayed by the globus pallidus, which has not been destroyed, it is suggested that the projections reported in this cat are in part or all of cortical origin. Gurdjian reported in normal cat material a strio-hypothalamic component within the medial forebrain bundle

which is said to arise, in part, from the head of the caudate nucleus. We would not like to assume such a component because the processes of the neurites from the caudate nucleus are indeed the shortest in the central nervous system.

Since the medial and lateral hypothalamic areas and the other nuclear masses of the hypothalamus have not shown terminal degeneration, it is suggested that a cortical projection through the internal capsule reaches the preoptic area as well as the supraoptic nucleus. The doubtful terminal degeneration found scattered within the paraventricular nucleus leads to the conclusion that this nucleus receives its heaviest projection via the septum or with a relay from the septum.

Of the midline nuclei, the nucleus reuniens and nucleus centralis medialis and lateralis, show a heavy projection. These nuclei of the midline group belong to that group of thalamic nuclei which are relatively constant in the mammalian scale and phylogenetically the oldest and therefore probably (Walker) concerned in the most primitive forms of sensibility receiving impulses especially from internal organs and viscera as well as from spinothalamic tract and medial lemniscus. Their efferent connections are mainly with the hypothalamus (Rousay and Mosinger) through five poorly defined thalamo-hypothalamic tracts as described by the above authors. Cortical efferents reach them, and therefore a possible reciprocacy must be considered. The vasomotor changes seen in thalamic lesions and the retention of certain localization of painful stimuli in hemidecortication acquires an anatomical basis.

The crossed cortico-thalamic fibers to nucleus commissuralis interanteromedialis, nucleus antero-medial and antero-ventral, which are

directly connected to the neocortex (cingulate gyrus), receive great importance as crossed cortico-thalamic fibers have never been reported though postulated.

The cortical projection from the frontal granular cortex to the neothalamic, large parvocellular portion of dorsomedial nucleus is also confirmed by the bouton method.

The conical shaped nucleus centrum medianum (centre median of Luys) with its small, oval or spindle shaped cells also receives a cortical projection. The nucleus shows a progressive development in ascending phylogeny and its fiber connections are totally obscure. The nucleus is thought to be in close relation with globus pallidus though such connections appear to lack teleological significance for the pallidus is phylogenetically an old part of the central nervous system and the oldest part of the striatum.

The importance of this nuclear mass in relation to trigeminal pain and lobectomy acquires a significance since it has been reported that trigeminal fibers end in the centrum medianum (Marchi studies, Ranson et al and Clarke et al). The above has been confirmed by us through the application of the bouton method which results are to be reported later (cat 32A).

With midline destruction of the inferior hypothalamic decussation in cat 26A, boutons have been found in supraoptic, suprachiasmatic and ventromedial hypothalamic nucleus, as well as in the supraoptic and infundibular region.

It has been thought that the inferior hypothalamic decussating fibers arise from various sources including the zana incerta, lenticular

nucleus, reticular nucleus of thalamus, paraventricular nucleus, subthalamus, entopeduncular nucleus, globus pollidum, supraoptic nucleus and geniculate bodies.

All of above areas or nuclei have been checked with the retrograde and bouton method and none of the above mentioned areas has given any conclusive evidence.

Boutons have been seen in the medial geniculate bodies and pulvinar of both sides.

Therefore, the inferior hypothalamic decussating fibers not only conduct towards the hypothalamus but also in opposite direction and therefore the hypothalamus is in synaptic relation to the highest subcortical association center, the pulvinar, which projects to the highest cortical association center, the posterior superior parietal lobule, the angular and supramarginal gyri of the cerebral cortex; as well as with the auditory reflex center, the medial geniculate body, whose cortical projection is well known.

The inferior hypothalamic decussation is made up of two components; the heaviest bundle is coarse, heavily myelinated and tortuous, and the smaller, scattered and fine unmyelinated fibers.

The first component does not seem to synapse in the hypothalamus as these fibers, undergoing secondary (Wallerian) degeneration are easily traced to the region of basis pedunculi, while the second component seems to be in synaptic relation to the hypothalamic centers.

Which of the two components sends fibers to the geniculate and pulvinar is difficult to report at present for the fine unmyelinated fibers are extremely diffused and easily lost in their ventro-lateral course.

Cats 26AB and 27A

Cats 26AB and 27A have received an extensive lesion involving the midline nuclei of the thalamus. In cat 26AB the lesion extends from nucleus periventricularis anterior to nucleus reuniens posteriorly, while in cat 27A, the lesion extends to posterior aspect of dorsomedial thalamic nucleus.

Terminals (free and ring type of boutons) have been found within the medial hypothalamic area (the medial periventricular nucleus). The greatest distribution of the ring type of ending is seen in the infundibular region. The rest of the extensive periventricular nucleus shows primarily the free ending type of degeneration. The fibers of conduction, which are beaded and with degrees of vacuolation, are seen to arise from dorsal areas (the midline nuclei) and not, as one might postulate, from anteriorly placed areas, septum or cortex, which travel subependymally. The latter analysis is in part justified as in both cats (26AB and 27A) the anterior periventricular nucleus has been destroyed and therefore a partial destruction of the anterior component of the periventricular system.

It is also observed that beaded fibers are seen travelling with the paraventricular-hypophysial fasciculus. These fibers can only be postulated because they appear to be fibers belonging to the inferior thalamic peduncle, which, in part, arises from the midline nuclei, and which is undergoing secondary (Wallerian) degeneration. These heavily myelinated fibers which are in contrast to the non-myelinated periventricular component from the midline nuclei, are easily detectable in the colloidal silver preparations as they become selectively overly impregnated.

Cat 27A has produced terminal degeneration in the medial hypothalamic area (periventricular nucleus), paraventricular nucleus of hypothalamus and the anteriorly placed nuclei of septum. The terminals within the medial periventricular hypothalamic area confirm the findings in cat 26AB and therefore further proof of the extensive thalamo-hypothalamic tracts as reported by Roussy and Mosinger which have been extensively discussed in the literature.

Terminals of the ring type have been observed in the paraventricular hypothalamic nucleus. These efferents to this supposedly subcortical vegetative center arise also from the medial magnocellular portion of the dorsomedial thalamic nucleus. Finely myelinated fibers with primary degenerative changes within them are seen streaming ventrally within the internal medullary lamina towards the neurites of the paraventricular nucleus. This connection has long been suspected from clinical data and Le Gros Clark further suggested that the dorsomedial nucleus appears to be developed from the nuclei of the midline and therefore is closely related to hypothalamic vegetative mechanisms.

On the extensive terminal degeneration observed in the medial and lateral septal nuclei (cats 26AB and 27A) it can only be speculated because these long axons are difficult to follow to their source of origin.

Since the midline nuclei are involved in this system of connections, it appears likely that the periventricular system of fibers are primarily involved which not only conduct ventrally (hypothalamus) and posteriorly (stem) but also to anteriorly placed structures (septum).

CHAPTER V

SUMMARY

An absolutely reliable colloidal silver method is presented which has been employed for staining and identifying degenerated myelinated and unmyelinated fibers as well as free and bouton type of terminals. This technique has revealed the substratum for the following connections:

1. Cortico (septo) paraventricular tract which courses with the medial forebrain bundle and with the anterior periventricular fasciculus.
2. Cortico-infundibular tract coursing principally within the periventricular area destined to the dorsomedial and ventromedial hypothalamic nucleus.
3. Paraventriculo-cortical tract. The axons arise from the large magnocellular neurites of the paraventricular nucleus destined to the frontal cortex which course through the septum.
4. Thalamo-paraventricular tract. The bundle arises from the dorsomedial thalamic nucleus and courses with the scattered bundles of the periventricular fibers.
5. Thalamo-periventricular tract. The fibers arise from midline nuclei of thalamus which are in synaptic relation to the periventricular nucleus of the hypothalamus. These fibers travel with the periventricular fasciculus.
6. Medullary-paraventricular tract. A compact bundle of non-myelinated fibers which reach the antero-lateral aspect of the paraventricular nucleus coursing with fibers of the internal medullary lamina.

7. Cortico-supraoptic tract. This capsular tract courses with the medial forebrain bundle to reach the anterior aspect of supraoptic nucleus.
8. Cortico-hypothalamic tract to the preoptic and lateral hypothalamic area also coursing with medial forebrain bundle.
9. Fibers of inferior hypothalamic decussation to supraoptic, suprachiasmatic and ventromedial hypothalamic nucleus.
10. Thalamo-hypothalamic tract. It arises from midline nuclei to be distributed to the infundibular area.

The following connections were confirmed also:

1. Cortico-thalamic fibers to large lateral parvocellular portion of dorsomedial thalamic nucleus.
2. Cortico-thalamic fibers to principal nuclei of the midline of the thalamus.
3. Cortico-thalamic fibers to controlateral anterior thalamic nuclei.
4. Cortico-thalamic tract to nucleus centro medianum.

APPENDIX

MICROPHOTOGRAPHS OF NORMAL AND DEGENERATING FIBRILS AND TERMINALS

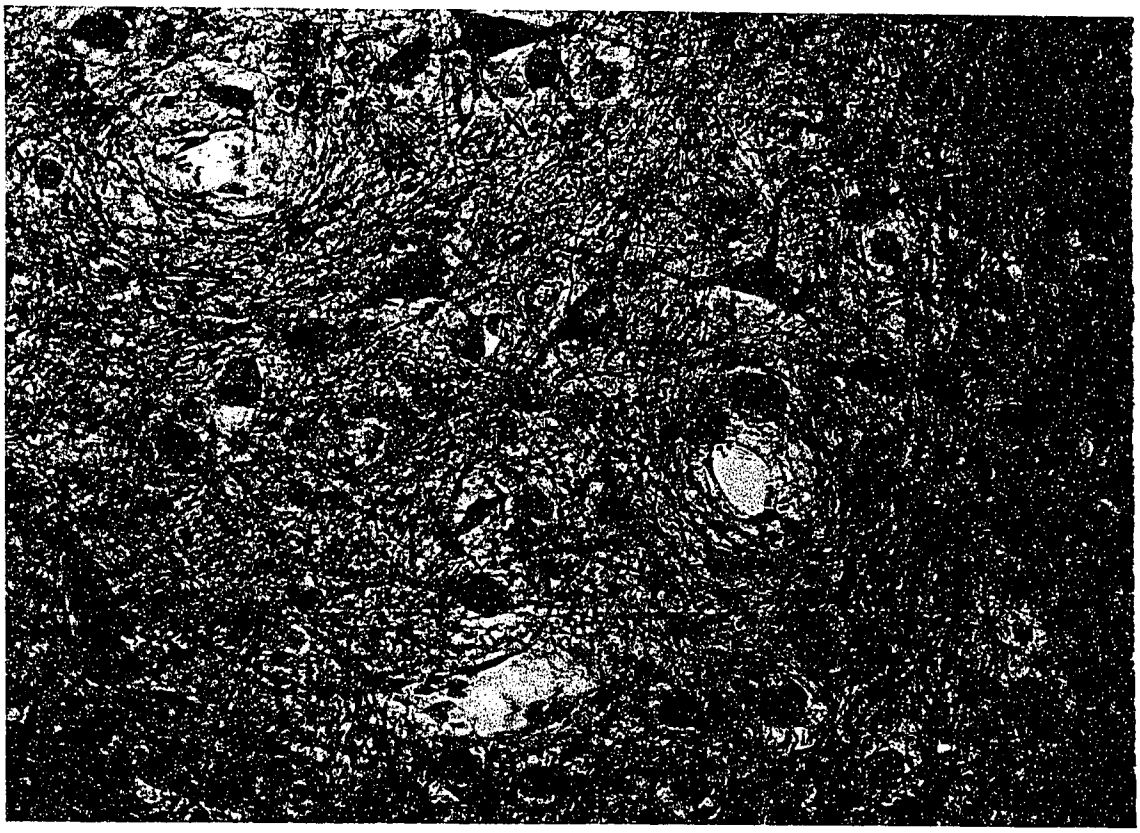

Fig. 1.— Paraventricular nucleus. Zenker acetic fixed tissue. Observe the extremely fine neurofibrils which form a mesh among the neurites. The fibrils, which are black to ordinary light, contrast well with the colorless background which is devoid of precipitated colloidal silver. The large neurites, with all its processes also stand out. The mixed cellular elements of the paraventricular nucleus is apparent also. X480.

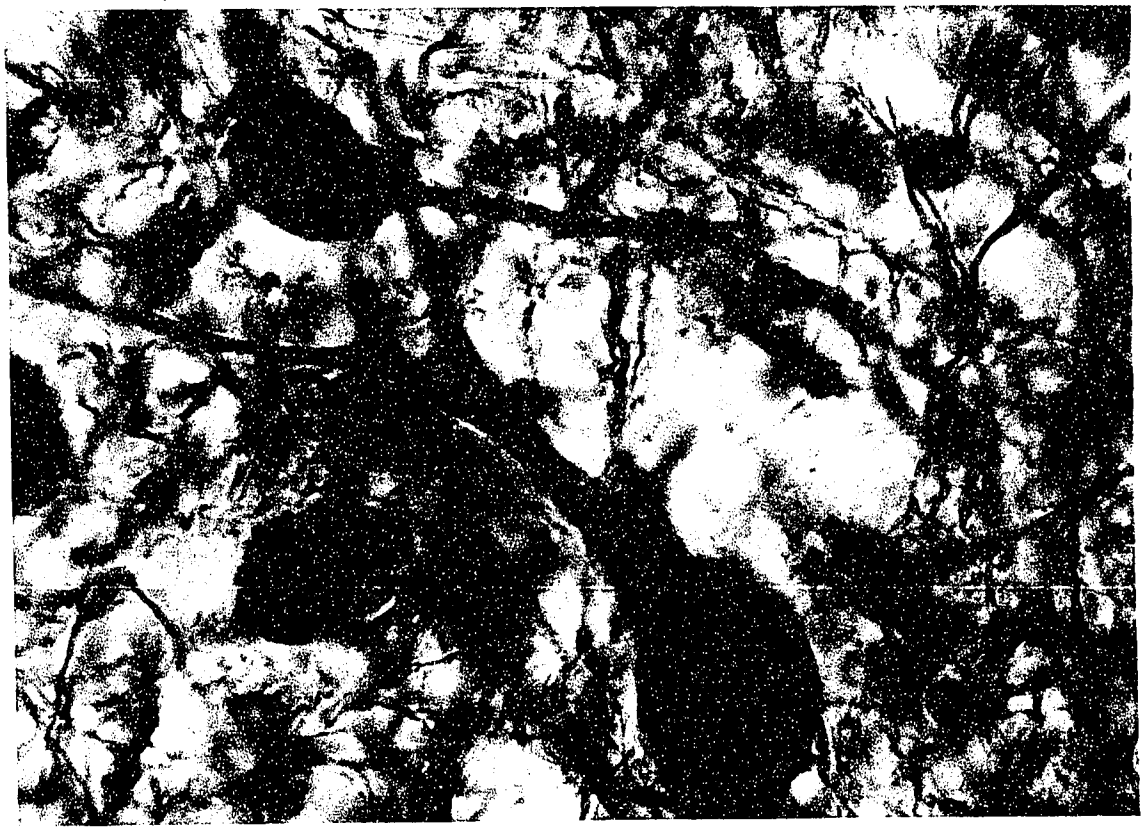

Fig. 2.— Magnocellular element of medial portion of paraventricular nucleus. Zenker acetic fixed tissue. The neurite shows degrees of granulation which is most apparent within the bifurcating dentrite. X1000

Fig. 3.— Paraventricular nucleus. Zenker acetic fixed tissue. Observe the fine bundle of fibrils coursing from the direction of internal medullary lamina (left) to a medial and ventral direction to reach the dorso-medial aspect of the nucleus. X120

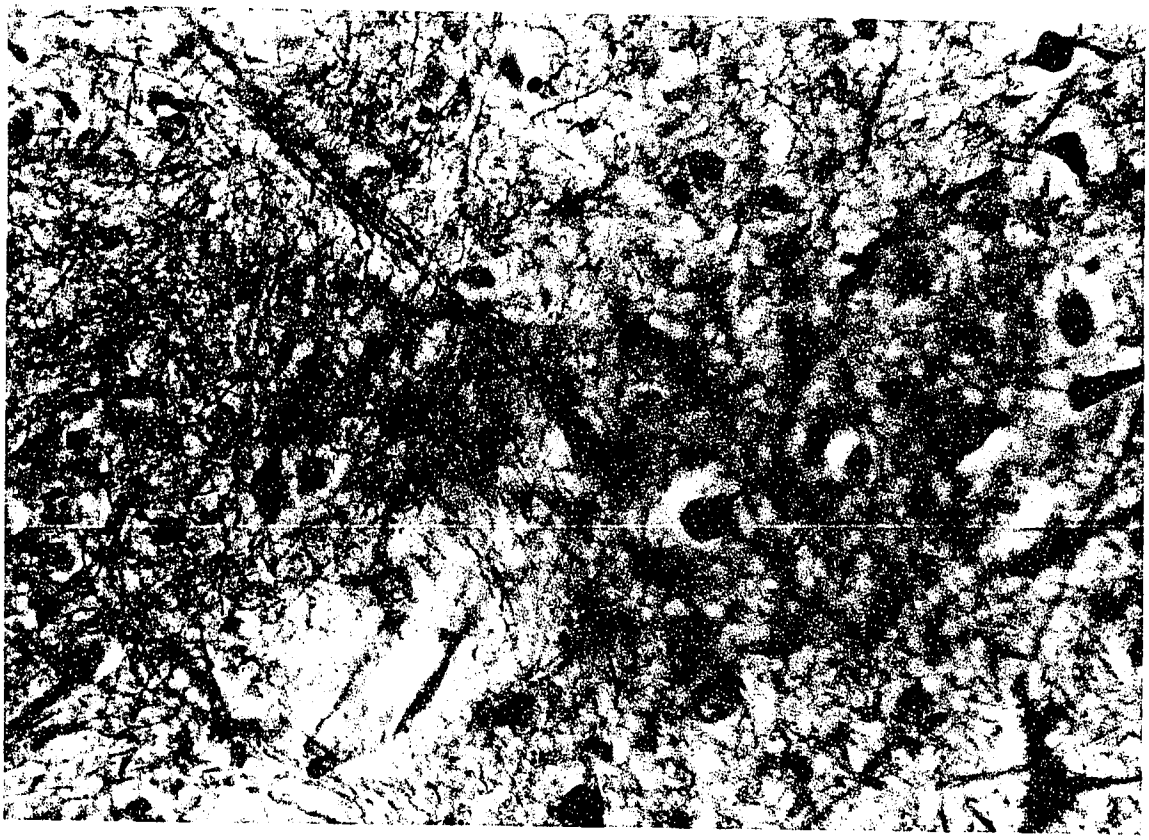

Fig. 4.— Paraventricular nucleus. Zenker acetic fixed tissue. The bundle of Fig. 3 is seen at a higher magnification. Observe the fine network of neurofibrils which permeate the nucleus itself. X420.

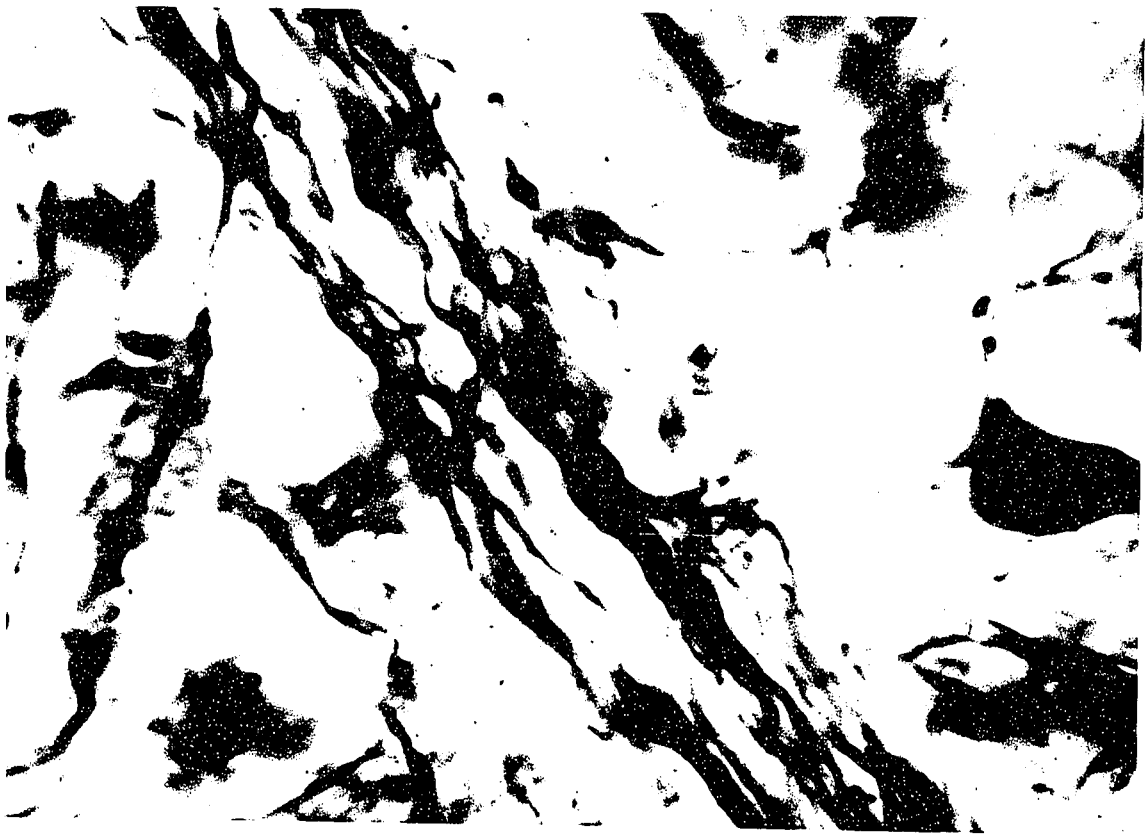

Fig. 5.— Paraventricular nucleus. Zenker acetic fixed tissue. The small bundle of fibers seen in Figures 3 and 4 is highly magnified to demonstrate its neurofibrillar make-up. Not all the neurofibrils are in focus; neither is the tissue around the tract. X1600

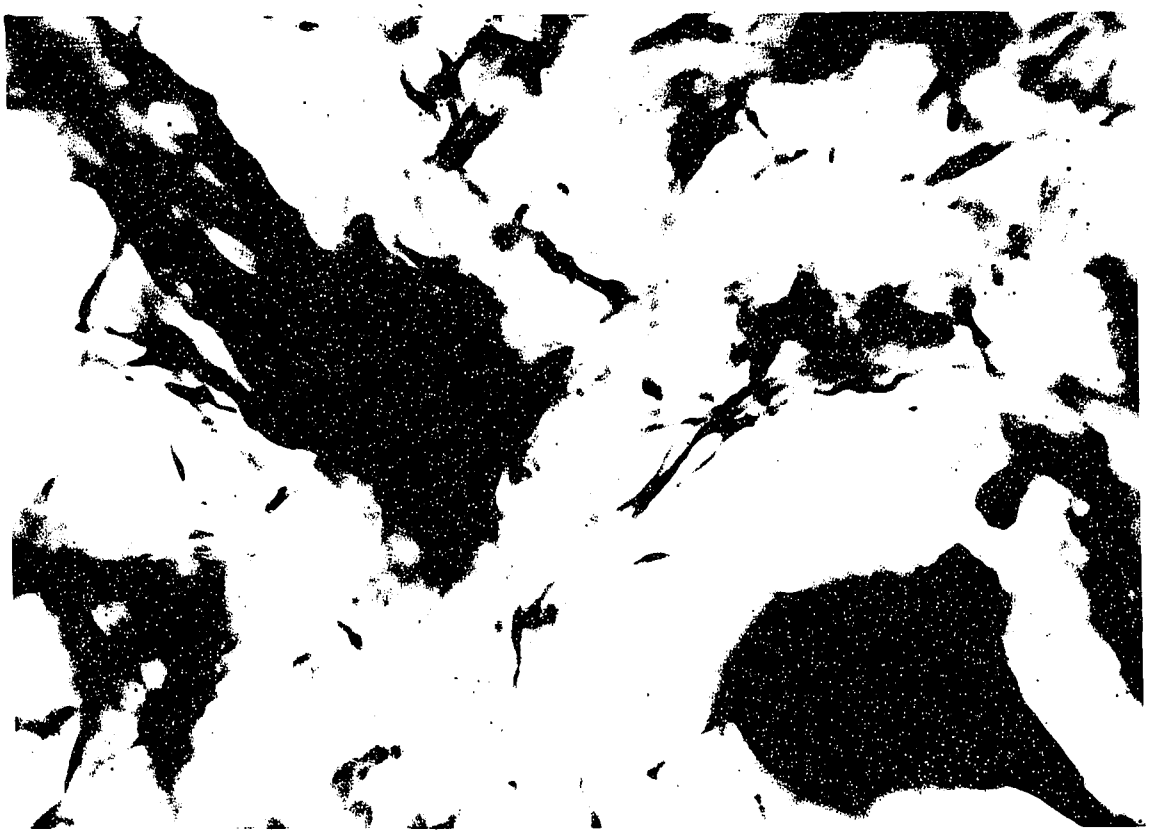

Fig. 6.— Paraventricular nucleus. Zenker acetic fixed tissue. The more superficial fibrils of the bundle are seen terminating at the outer margin of a large cell. The deeper aspect of the cell (to the right of photograph) and of the bundle is out of focus. The fascicle would explain the enormous amount of boutons seen within the nucleus. X1600

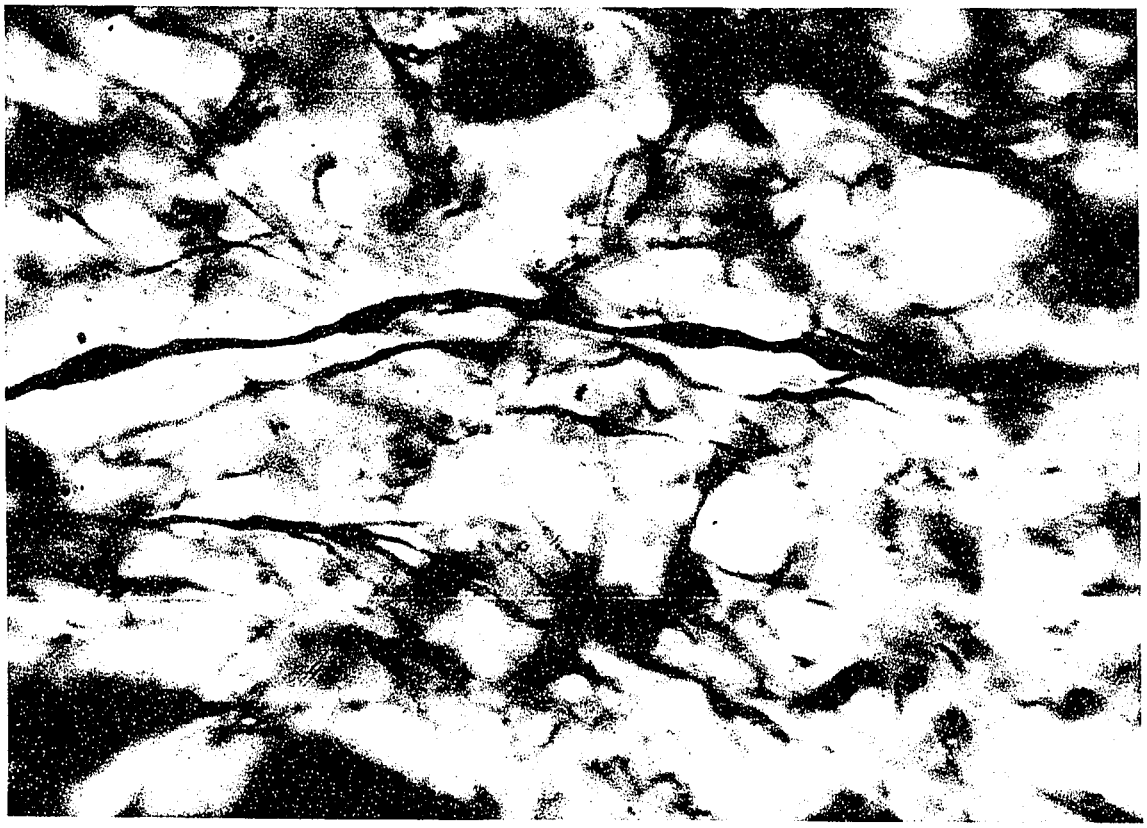

Fig. 7.— Paraventricular nucleus. Zenker acetic fixed tissue. The magnocellular portion of antero-medial aspect of nucleus. Disrupted the cortico- (septo)- paraventricular tract. The beaded and swollen fiber at center of picture might well be fibers of the paraventriculo-hypophysial tract since it is travelling with that fasciculus or cortico-septo-hypothalamic fibers to dorso-medial or ventro-medial hypothalamic nuclei or cortico-septo-infundibular fibers which have travelled with the periventricular system of fibers. X1600

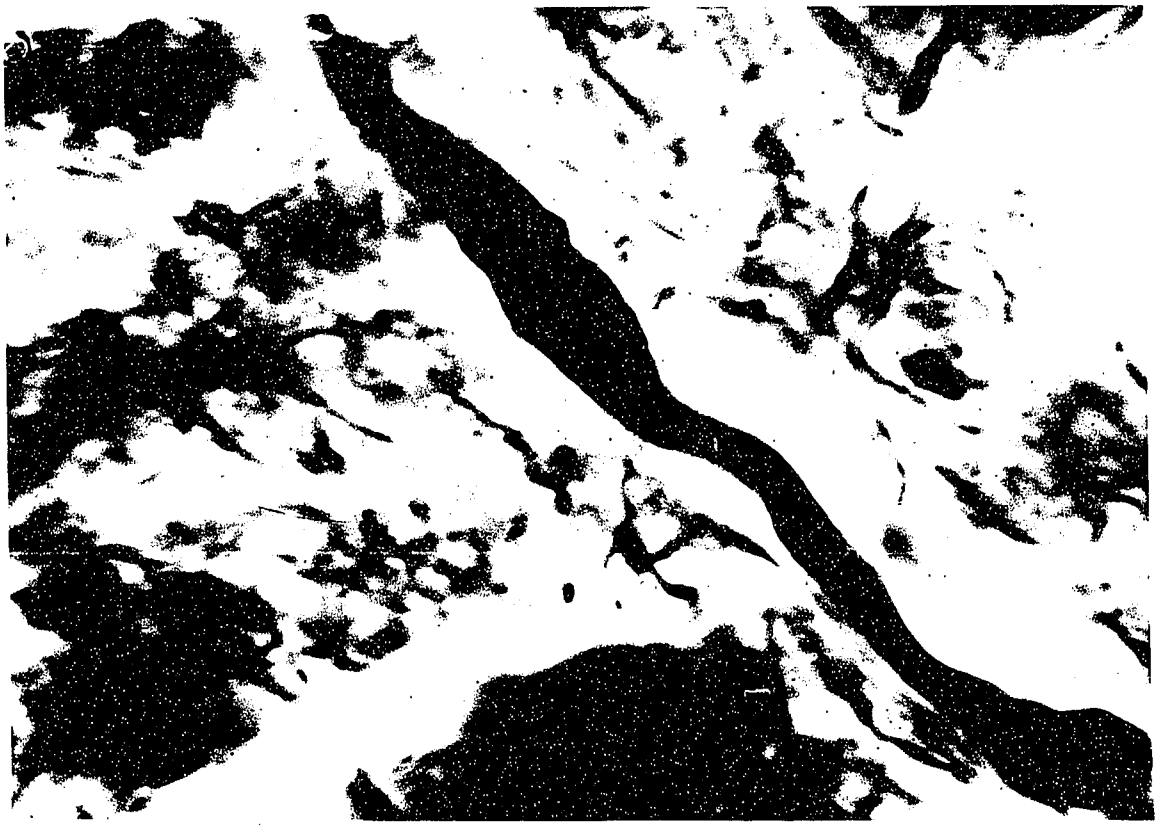

Fig. 8.-- Advanced secondary (Wallerian) degeneration of a myelinated fiber of thalamic origin coursing with the unmyelinated fibers of the paraventriculo-hypophysial tract which lies to the left and is out-of-focus. Observe that the fiber is tortuous, vacuolated and extremely argentophilic. The neurites are out-of-focus also. X1600

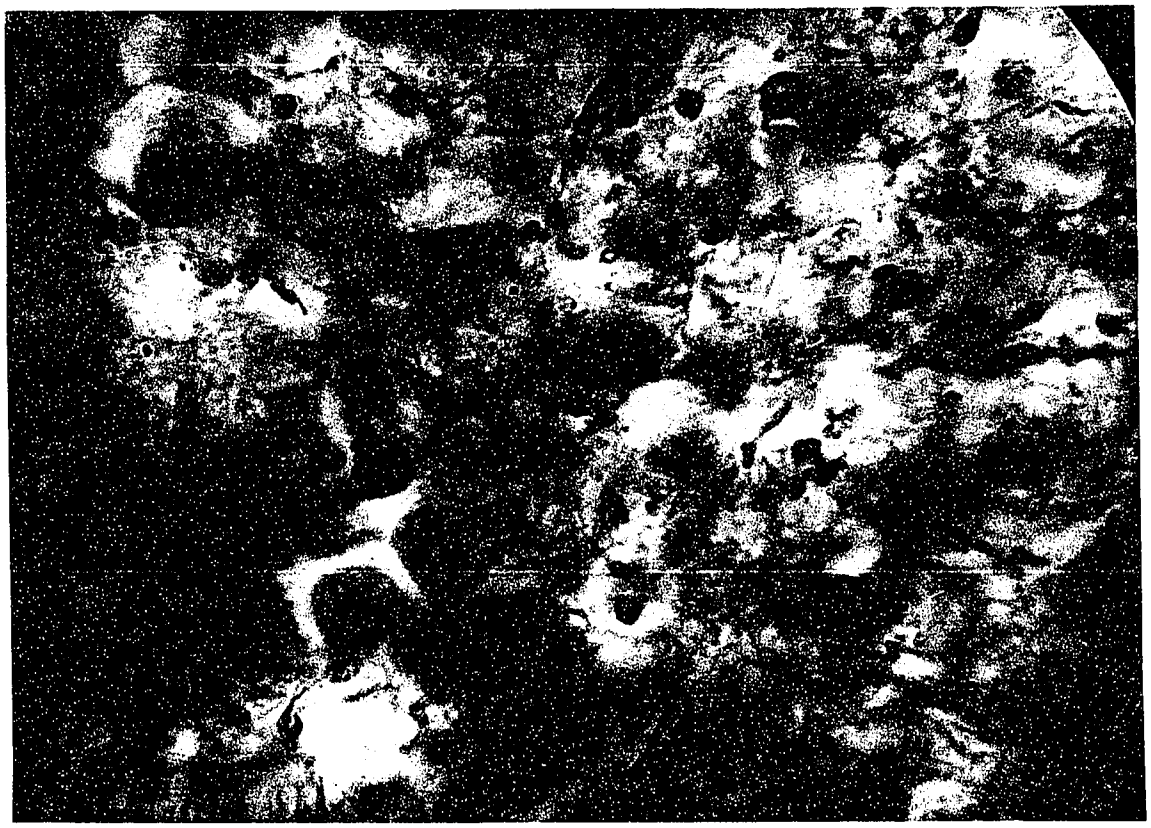

Fig. 9.— Terminal degeneration within the substance of para-ventricular nucleus due to electrolytic destruction of dorso-medial and median nuclei of thalamus. Rings and fibrillar fragmentation is apparent.

Acetone fixed tissue. Post-operative period of five days. X1600

Fig. 10.— Supraoptic nucleus. Normal. Acetone fixed tissue. Observe that in acetone fixed tissue the neurites do not stand out as well as they are brought out in modified Zenker acetic fixed tissue which gives a semi Golgi preparation. The neurofibrillar network of supraoptic nucleus is most apparent. The coarse fibers seen at left upper corner belong to optic system. X1000

Fig. 11.— Normal supraoptic nucleus. Acetone fixed tissue. Rarely seen, few unusually long fibrils reaching the supraoptic nucleus from a dorsal direction. Observe that the long non-myelinated fiber, at its mid region, shows a constriction, supposedly the node of Ranvier. The neurofibrillar network on the left side of photograph is slightly out of focus.

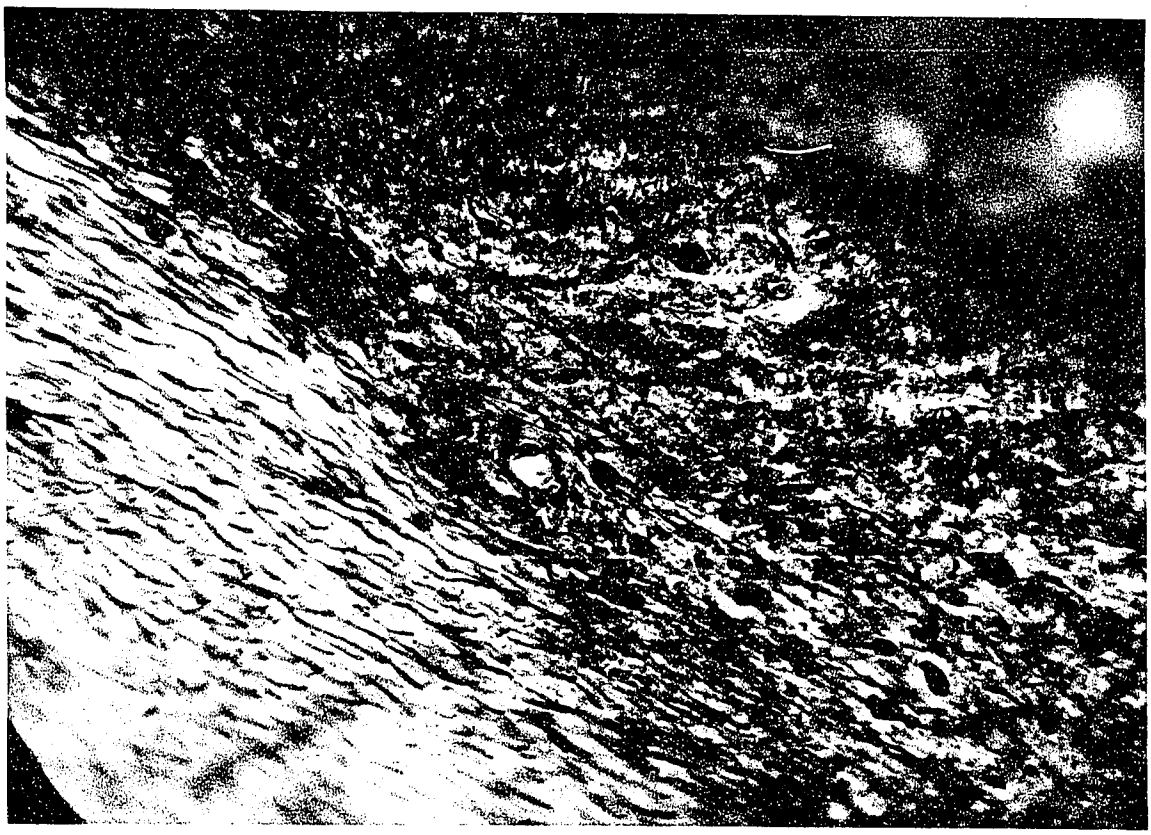

Fig. 12.— Degeneration proximal to and within supraoptic nucleus. Observe the gigantic retraction bulbs of Cajal which permeate the area of the nucleus. Among the bulbs are found terminal rings (see Fig. 21). The heavy bundle of fibers in the left inferior corner is the optic chiasma (tract). The inferior hypothalamic decussation was severed by knife lesion. Post-operative period of five days. Acetone fixed tissue. X1000

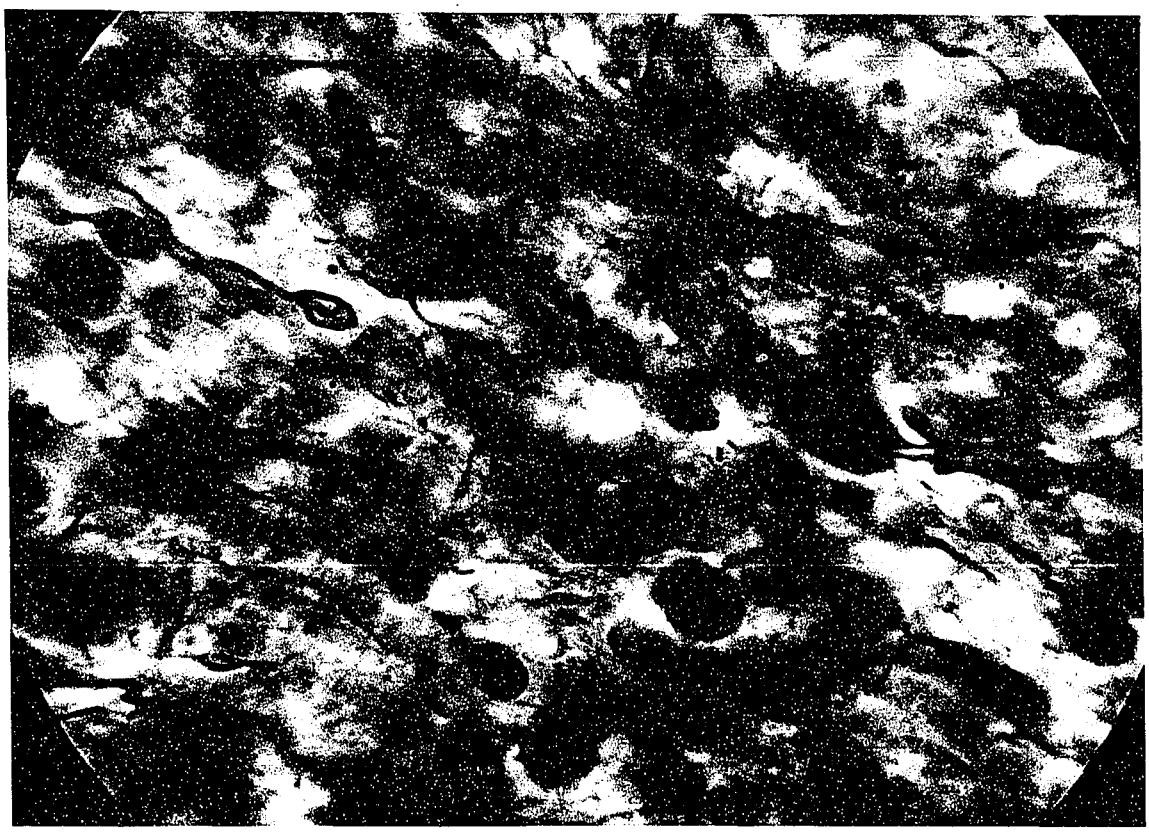

Fig. 13.— Terminal bulbs and rings within the substance of supraoptic nucleus. The terminal rings are apparent while the bulbs (dark-stained bodies) are out of focus. Tortuous and broken fibers are also seen. Knife lesions of inferior hypothalamic decussation. Post-operative period of five days. Acetone fixed tissue. X1600

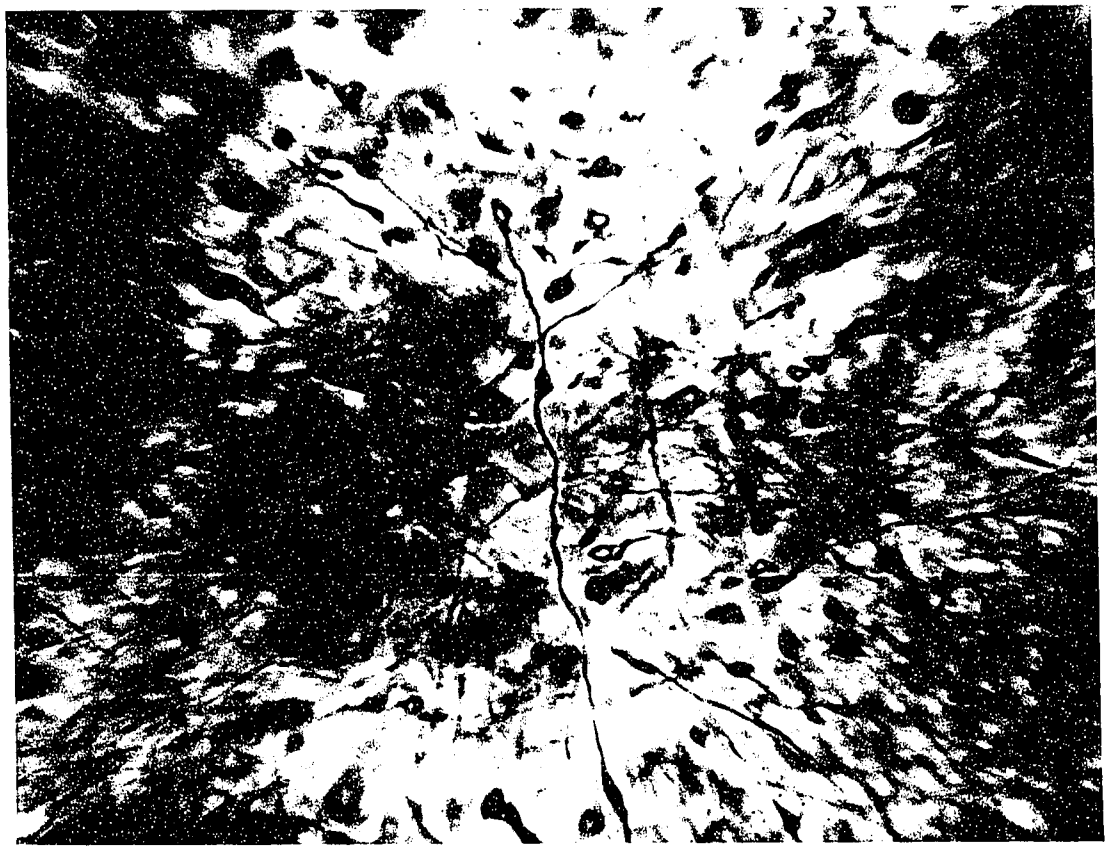

Fig. 14.— Median mammillary nucleus. Acetone fixed tissue. Observe the numerous terminals which are in the form of rings and bulbs. The fiber at center of figure has three swellings which appear as rings. The fibrils are swollen, beaded, and at various other stages of disintegration. Some of the above structures are out of focus. The neurites are completely overshadowed by the plexus of terminals. The fornix was destroyed electrolytically at region of anterior commissure. X1600

Fig. 15.— Capsular region of medial mammillary nucleus. Acetone fixed tissue. Terminal rings and bulbs of various size and shape are apparent. The fibrils are in various stages of fragmentation and the neurites are obstructed by the terminal plexus of terminals. Fornix was destroyed at region of anterior commissure. X1600

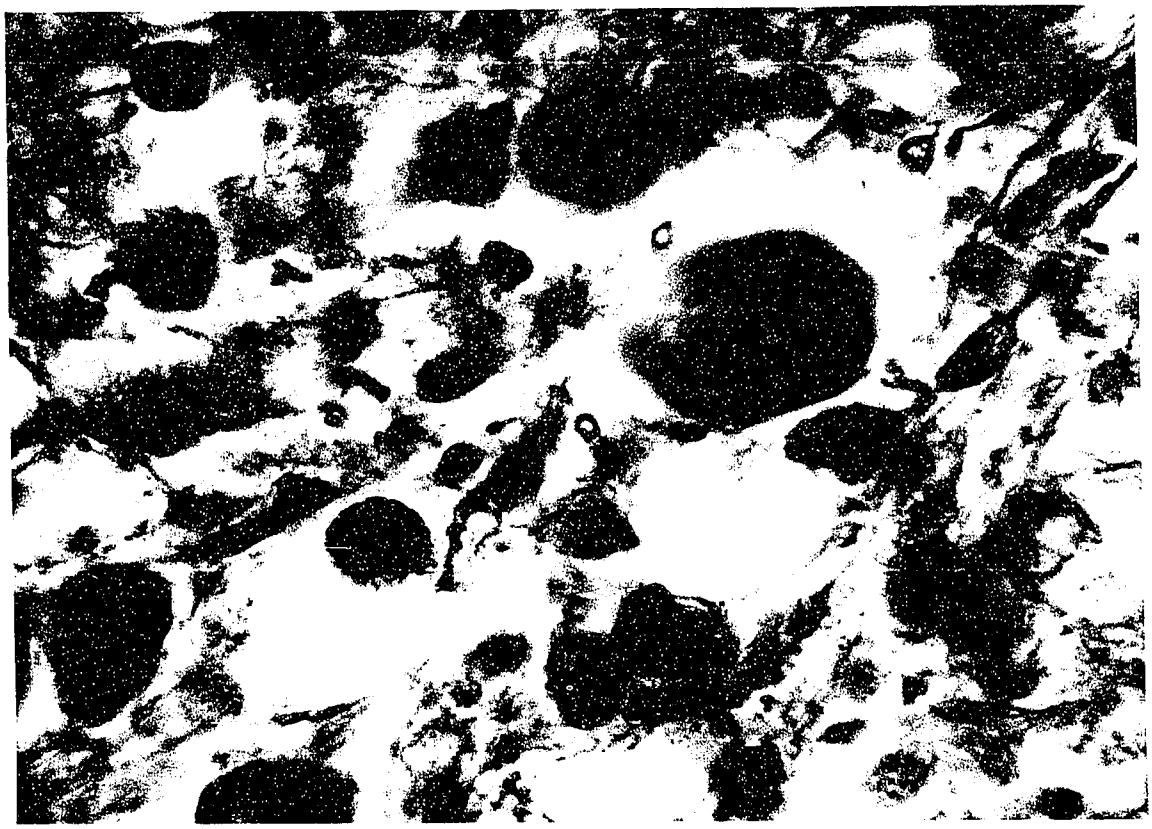

Fig. 16.— Pre-mammillary region. Acetone fixed tissue. The concentric large structures are nuclei and boutons which are seen around a clear perinuclear space as the cell is out-of-focus. Other boutons (rings) are present but out-of-focus. X1000

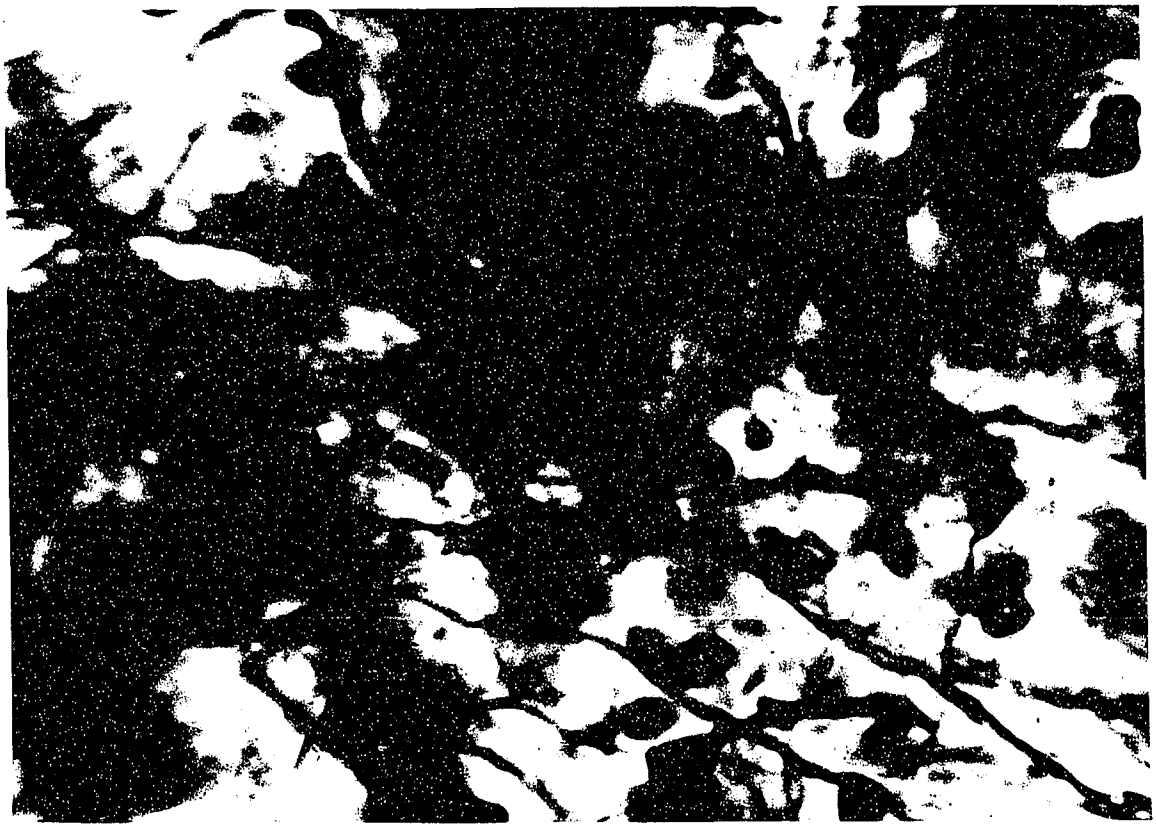

Fig. 17.— Medial habenular nucleus. Acetone fixed tissue. The ring and bulb type of terminals is apparent. The neurofibrils are in various stages of disintegration. The relationship of terminals to neurites is not apparent as the cells are out of focus. The darkly stained, large, round or oval structures are the nuclei of the neurites. The nuclei are excessively argentophilic when in stages of disintegration. Normally, the nuclei are stained lightly. This would indicate transneuronal possibilities. The stria habenularis was destroyed electrolytically at preoptic region. X1600

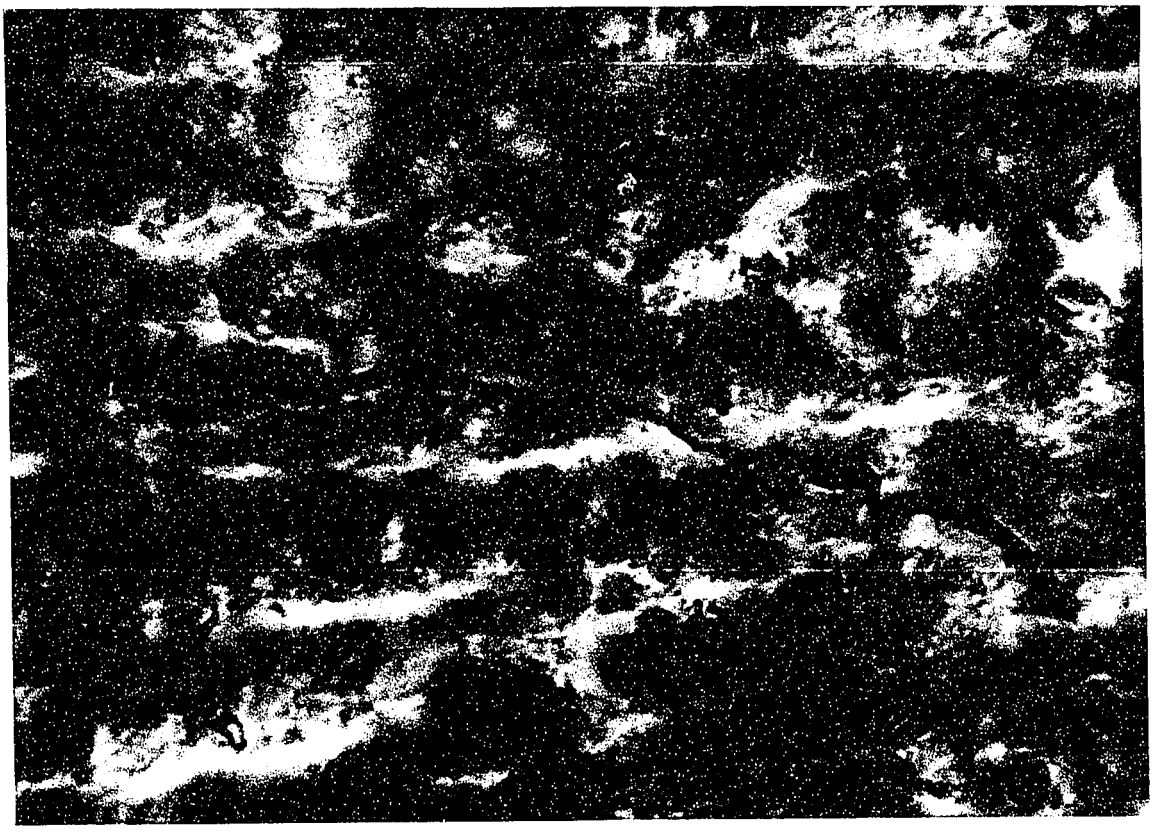

Fig. 18.-- Preoptic region. Acetone fixed tissue. Bulbs and rings are apparent with a swollen and tortuous fiber. X1000

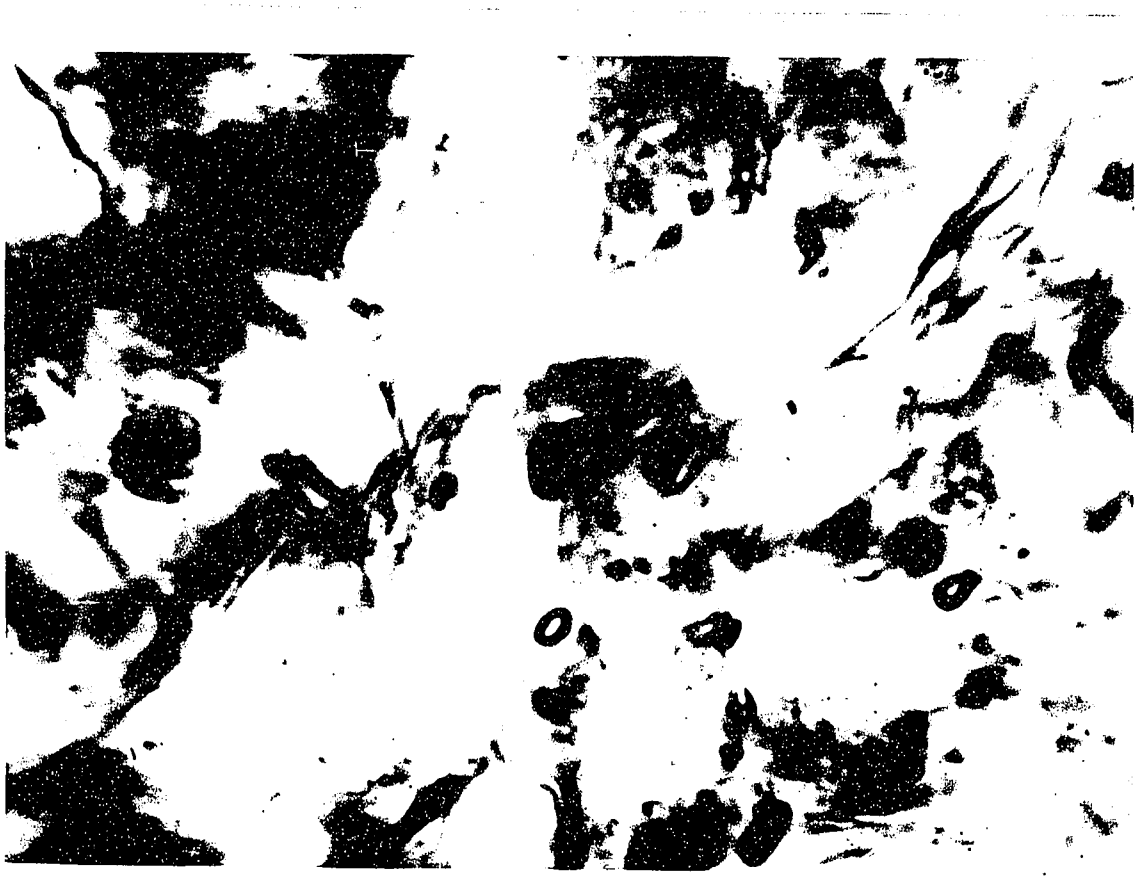

Fig. 19.— Medial preoptic area. Acetone fixed tissue. Due to the short neurons, the boutons are few and scattered. The above photograph shows an unusual concentration (4 in focus) at such high magnification. The fibrils are in part re-absorbed. X1600.

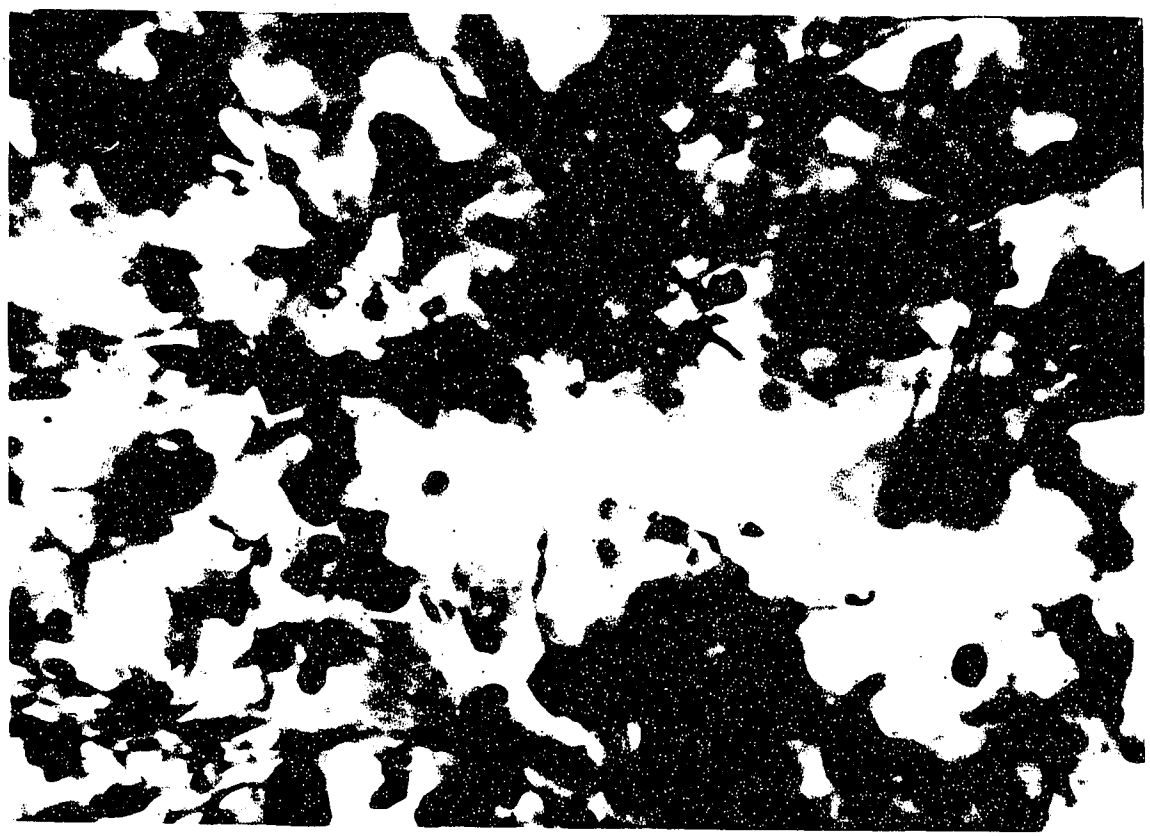

Fig. 20.— Suprachiasmatic nucleus. Acetone fixed tissue. Rings and bulbs of various morphological variations are apparent. The fibrils have practically all disintegrated. The nuclei are darkly stained and stand out among the boutons. X1600

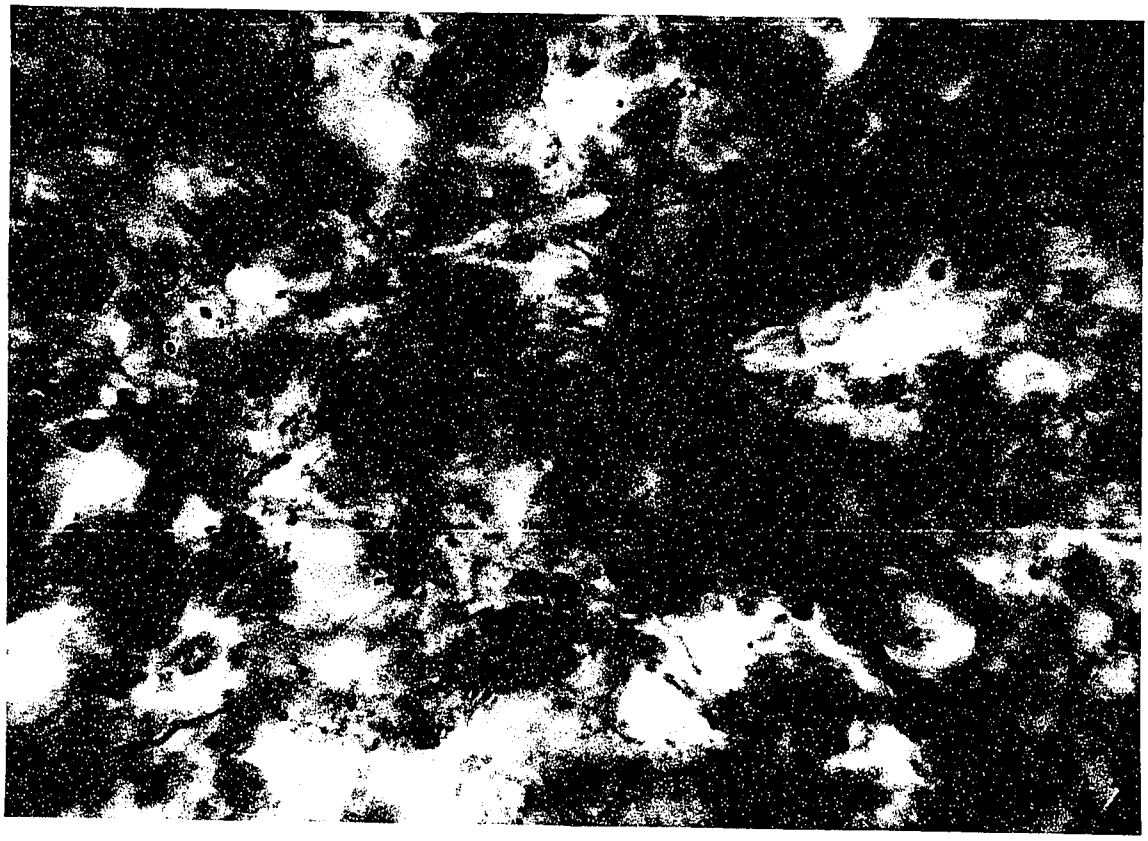

Fig. 21.— Abundant terminals of the ring type within the suprachiasmatic nucleus due to lesions of inferior hypothalamic decussation. Acetone fixed tissue. Post-operative period of five days. X1600

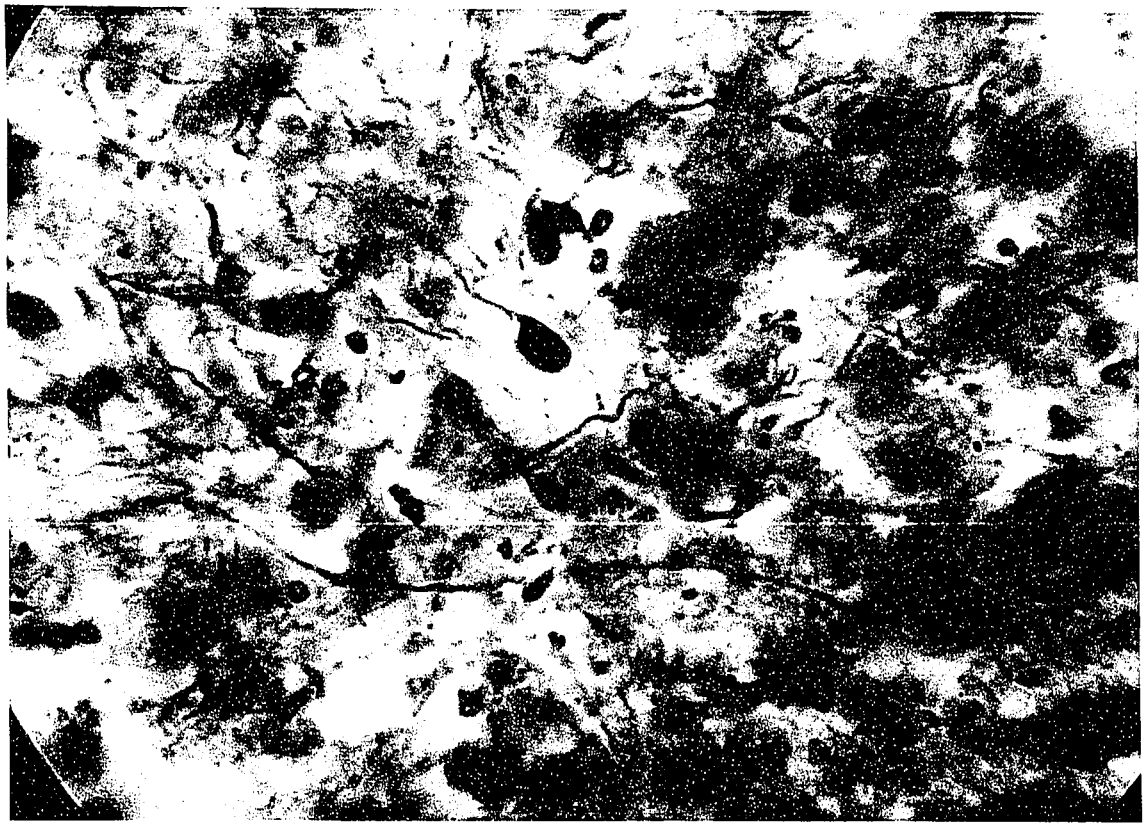

Fig. 22.— Terminal degeneration within the ventro-medial hypothalamic nucleus due to lesion of inferior hypothalamic decussation. Rings are abundantly scattered among the neurites. Acetone fixed tissue. Post-operative period of five days. X1600

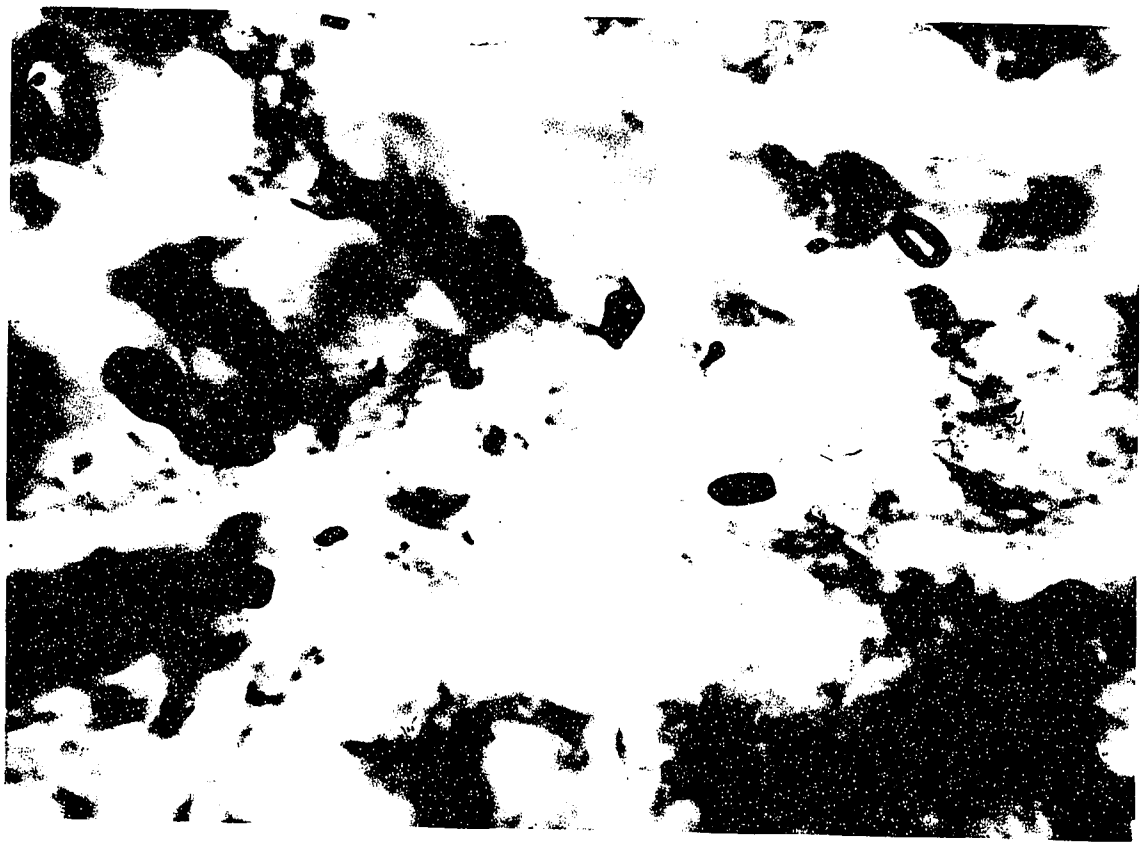

Fig. 23.-- Nucleus ventromedialis hypothalami. Acetone fixed tissue. Terminal rings are apparent. The nucleus is in an advanced stage of degeneration and therefore most of the fine unmyelinated neurofibrils have been re-absorbed which explains the clear space. The neurites are out of focus. X1600

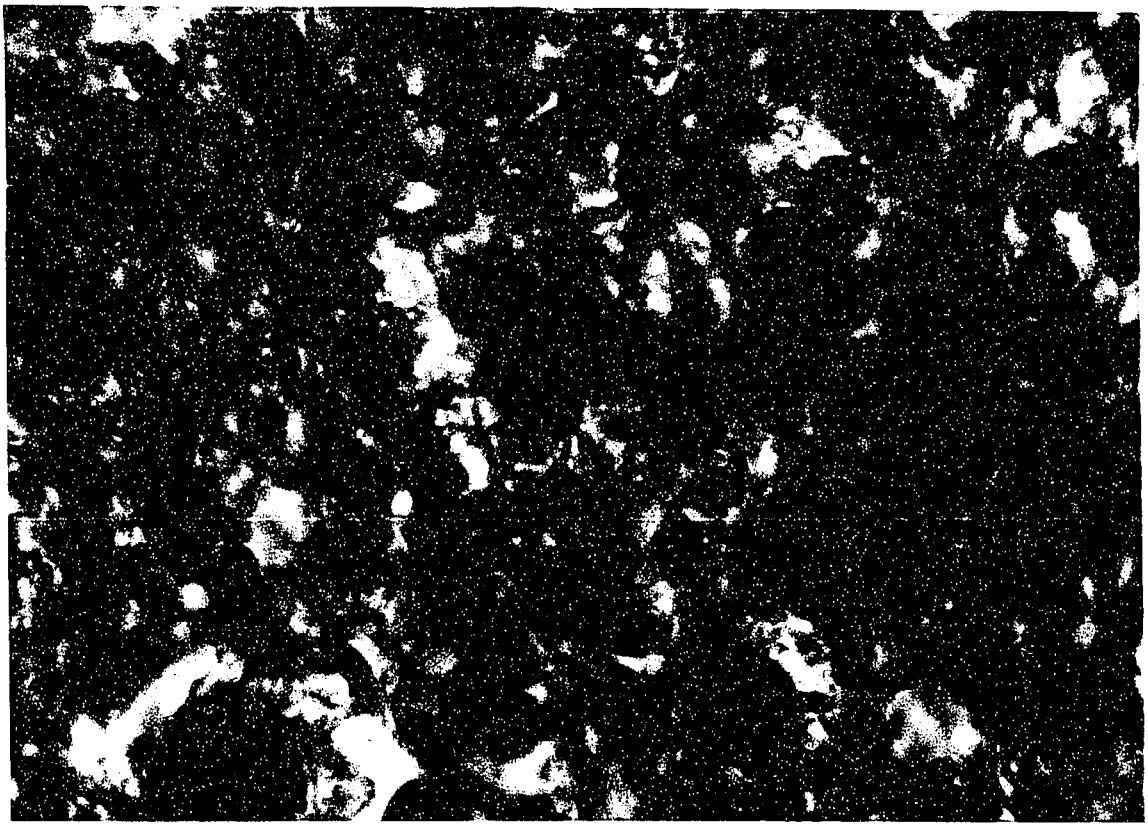

Fig. 24.— Nucleus Ventralis. Acetone fixed tissue. Advance terminal degeneration. Bulbs and rings are apparent. Fibers are in various stages of disintegration: beaded, swollen, tortuous and fragmented. X1000

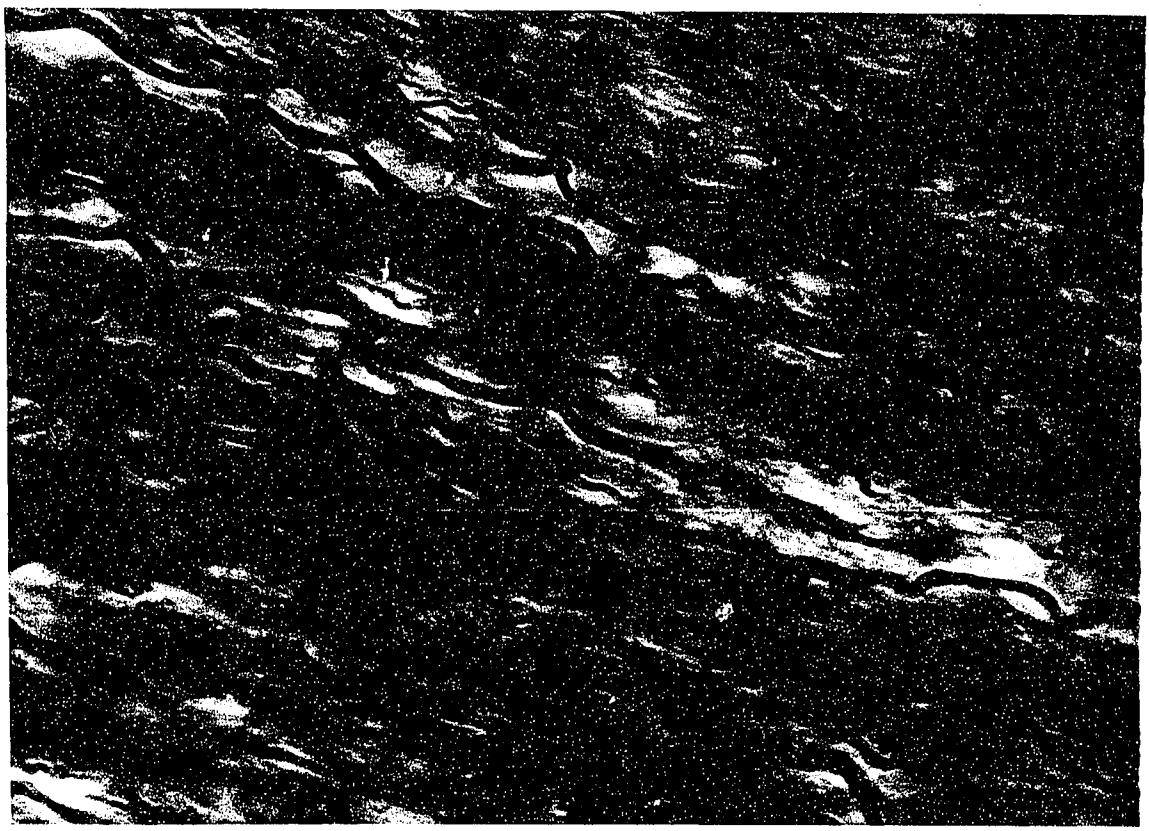

Fig. 25.— Intrafascicular fibers. Acetone fixed tissue. Four days after an extensive lesion of thalamus. Observe the thick, tortuous and swollen fibers which are undergoing secondary (Wallerian) degeneration. X1000.

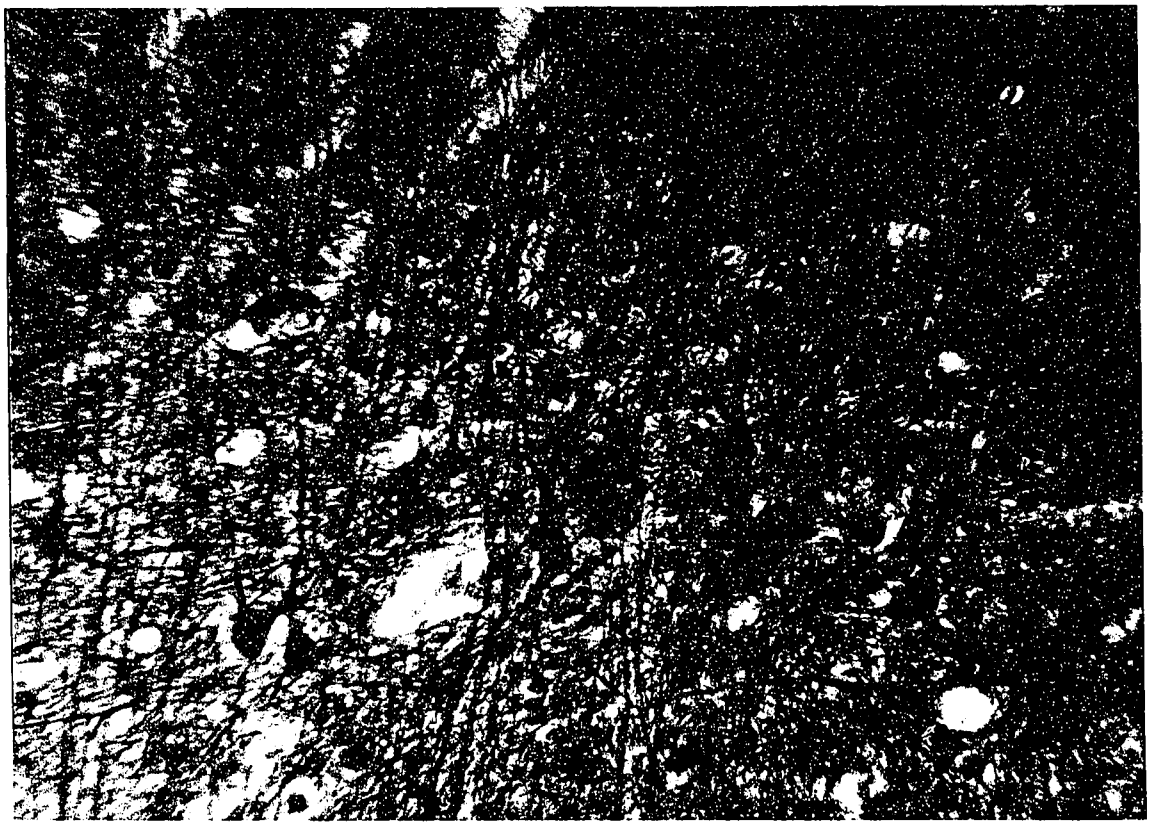

Fig. 26.— Normal cerebral cortex of cat fixed in modified Zenker acetic. Observe the neurofibrillar networks and the semi Golgi aspect of the neurites in layer V. Compare with Figures 27, 28 and 29. X516

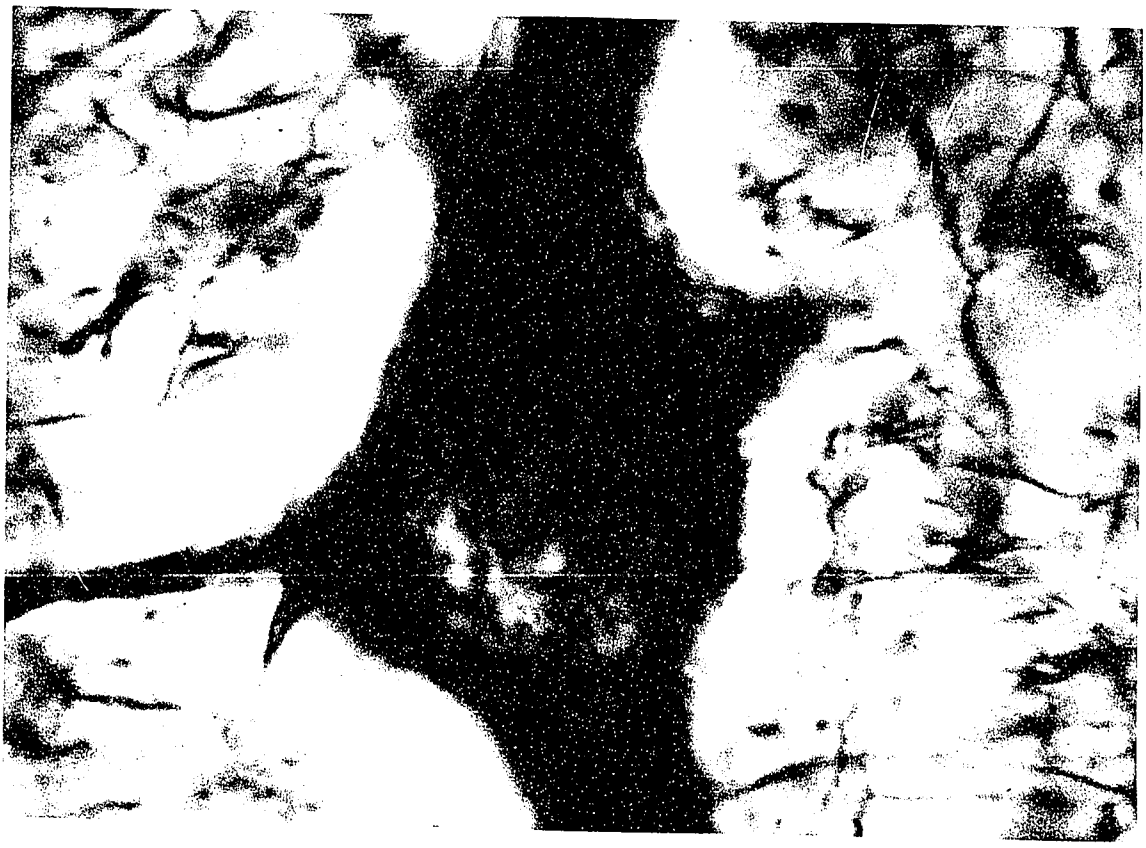

Fig. 27.— A multipolar cell of cerebral cortex of cat from layer V as seen in Figure 26 from Zenker acetic fixed tissue.

Observe the delicate neurofibrils that course through the perikaryon and the centrally located, lightly stained nucleus. The pericellular network around the cell is out of focus though seen in part at periphery of cell body. X1600

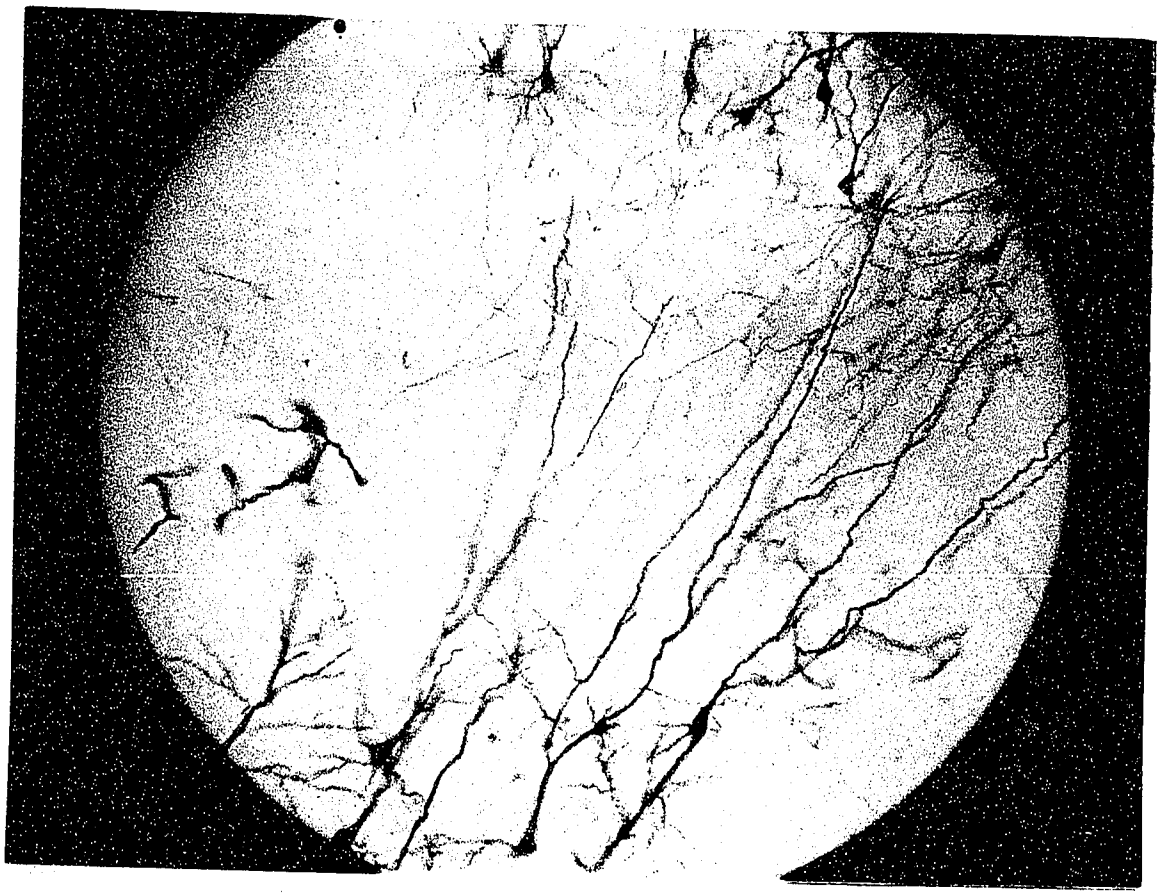

Fig. 28.— Golgi preparation on serial paraffin section from adult cat's brain which had been fixed in Zenker. The lower aspect of the photograph shows the dentrites of the large multipolar cells of layer V. The upper portion shows cells within layer III.

Compare with Figures 26 and 27. X1000

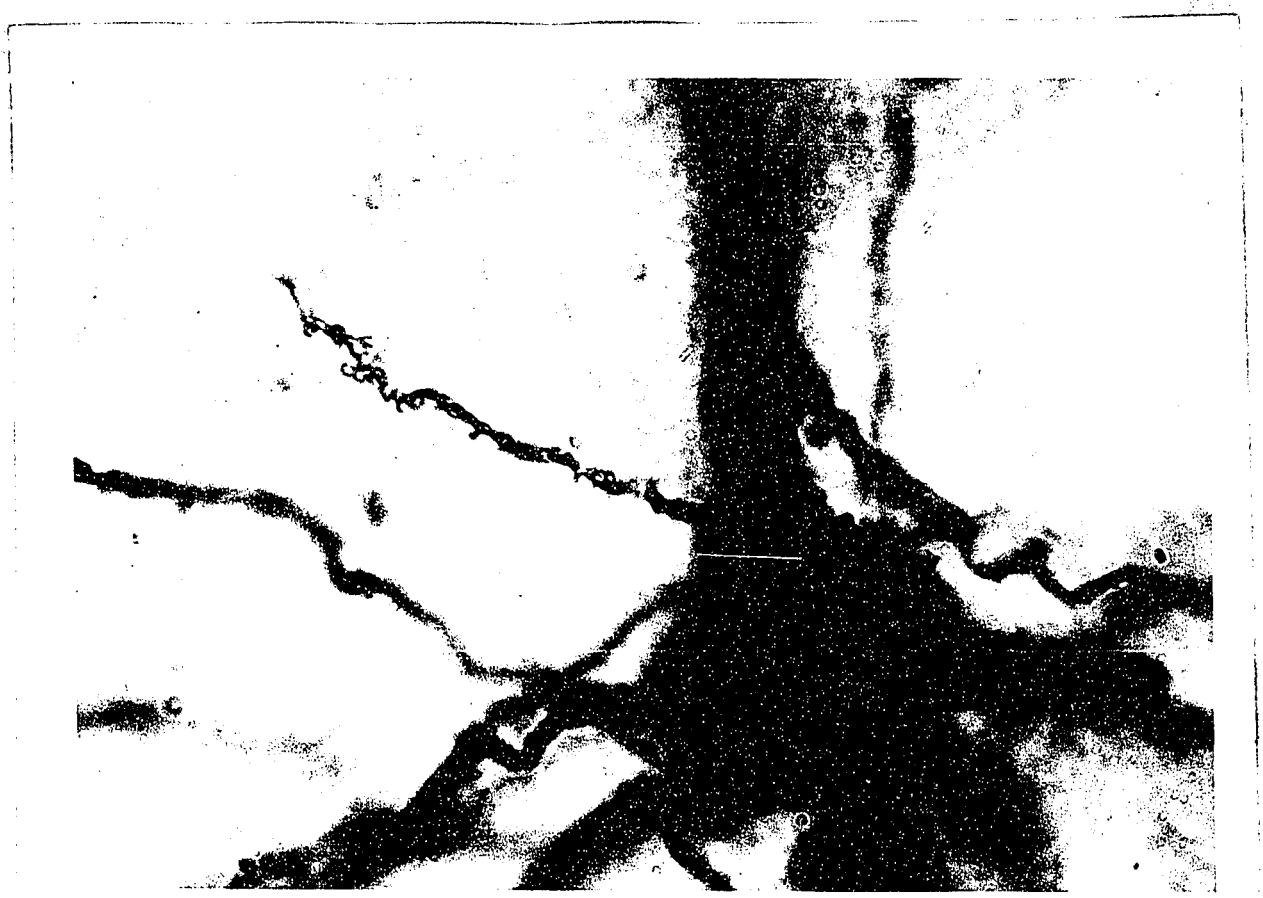

Fig. 29.— Higher magnification of cell body and dentrites of a multipolar cell of cerebral cortex of cat which had been fixed in Zenker and embedded in paraffin. Observe the spines on the surface of the dentrites which are supposedly analogous to terminals.

Some of the dentrites are out of focus.

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