Hypothalamic Projections to the Amygdala in the Cat: A Light and Electron Microscopic Study

by

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A THESIS

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CHAPTER I

Introduction

Historical Review

A complex of nuclei representing the archistriatum lies deep in the anterior lobe of the temporal cortex. This complex, the amygdala, has been recognized in various vertebrates from fish (Johnston, 1923; Droogleever-Fortuyn, 1961; Schnitzlein and Crosby, 1967; Schnitzlein et al., 1969), amphibian (Herrick, 1921; Hoffman, 1963; Schnitzlein et al., 1969) and reptile (Johnston, 1923; Schnitzlein et al., 1969) to mammal (Johnston, 1923; van der Sprenkel, 1926; Gurdjian, 1928; Young, 1936; Crosby and Humphrey, 1936; Fox, 1940; Crosby and Humphrey, 1941, 1944; Lauer, 1945; Jeserich, 1945; Brodal, 1947; Breathnach and Golby, 1954; Johnson, 1957a, 1957b; Koikegami, 1963; and Yu, 1969).

Johnston (1923) divided the nuclei of the amygdala into two groups according to their phylogenetic and ontogenetic development. The corticomedial group, composed of the cortical, medial and central nuclei as well as the anterior amygdaloid area and the nucleus of the lateral olfactory tract, was found to constitute the older part of the amygdala, whereas the basolateral group, consisting of the basal and lateral nuclei, were

considered to be of more recent origin. Fox (1940) classified the anterior amygdaloid area and the nucleus of the lateral olfactory tract as a separate group because of their anterior position.

In the selachians, Johnston (1923) identified the amygdala in the lateral portion of the forebrain as a single mass of cells which received a few fibres from the lateral olfactory tract. However, in the more specialized teleost two distinct cell masses were found in the posterior portion of the lateral olfactory area (Droogleever-Fortuyn, 1961). These were referred to as dorsolateral and ventromedial nuclei. Because the dorsolateral nucleus occupies a position in relation to the piriform lobe anteriorly and the hippocampus dorsomedially, Droogleever-Fortuyn (1961) suggested that it is homologous with the basolateral portion of the amygdala in higher forms. In addition, this author considered the ventromedial nucleus homologous with the corticomedial group of nuclei because it receives fibres from the lateral olfactory tract and sends efferent fibres to the preoptic and hypothalamic areas via the stria terminalis. However, Nieuwenhuys (1962, 1965) stated that such a comparison with species of other classes cannot be made in this instance because the actinopterygian fish have specialized along their own evolutionary lines and the teleost

represents the end of this side-line of vertebrate evolution.

The amygdala of the amphibian, like that of the fish, was identified by following the fibres of the lateral olfactory tract (Herrick, 1921, 1933; Hoffman, 1963). No individual amygdaloid nuclei were identified in the urodele (Herrick, 1921, 1965), but a corticomedial and a basolateral portion were noted in the anuran brain (Hoffman, 1963). These subdivisions in the anuran have connections with the lateral olfactory tract and the stria terminalis which are similar to those found in the actinopterygian fish.

In the reptile, the cerebral hemispheres have enlarged and lengthened (Johnston, 1923) and the associated increase in the number of fibres in the internal capsule caused the fibres of the stria terminalis and its accompanying gray matter to arch dorsally. According to Johnston (1923) the gray matter in which these fibres lie forms the bed nucleus of the stria terminalis, which expands into the temporal lobe as the central and medial nuclei of the amygdala. These nuclei plus the cortical nucleus are derived from the primitive olfactory area found in selachians. This whole area in chelonia contains small cells and is connected with the parolfactory area and the

hypothalamus. But in addition to this area of small cells, Johnston observed a large celled area not found in the selachians. By following the development of this large celled area in the human embryo, he concluded that this nucleus as well as a lateral nucleus are formed later than the cortical, medial and central nuclei by an infolding and inward migration of cells from the piriform cortex at the amygdaloid fissure.

However, the infolding and cell proliferation from the piriform cortex have not been substantiated by more recent ontogenetic studies of the human embryo by Macchi (1951) and Humphrey (1968). These authors stated that all of the amygdaloid nuclei originate from the medial and the lateral striatal ridges. In addition the ontogenetic observations of Johnston, which indicated that the central nucleus is formed early, were not supported by the work of Humphrey (1968). According to this author all of the amygdaloid nuclei, with the exception of the central, appeared by the time the embryo reached a CR length of 20.7 mm. The central nucleus, however, could not be identified until the embryo had reached a length of 22.2 mm CR.

The basic nuclei of the amygdala that were described by Johnston in 1923 have since been described in many mammalian species. However, variations in their

position have been noted. These changes in the position of the nuclei are due to a medial rotation of the amygdala caused by the increased development of the temporal lobe in higher species. There are also variations in the size and secondary differentiation of the various nuclei. These variations are described below with special attention given to the nuclei in the cat since it is the animal used in this study.

Corticomedial Group of Nuclei

Johnston (1923) placed the cortical nucleus in the primitive group even though he noted that it is later modified by a migration of cells at the amygdaloid fissure. This fissure marks the boundary between the cortical nucleus and the cortex of the piriform lobe (figs. 2-4). A cortico-amygdaloid transition area has been described bounding the fissure in the rabbit (Young, 1936), man (Crosby and Humphrey, 1941), the monkey (Lauer, 1945), the rat (Brodal, 1947; Yu, 1969) and in the porpoise (Breathnach and Golby, 1954). Although it was not described in the cat (Fox, 1940), Humphrey (1968) noted that it is undoubtly present in all mammals.

The cortex-like layer of pyramidal cells in the cortical nucleus is more closely arranged in the lateral portion than in the more medial part, suggesting a secondary

Fig. 1. Drawing of the anterior amygdaloid area.

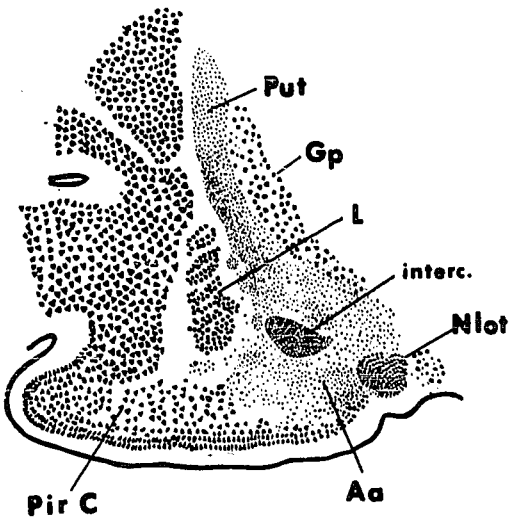
Fig. 2. Drawing of the amygdaloid nuclei at the rostral level of the amygdala.

Fig. 3. Drawing of the amygdaloid nuclei at about the middle of the amygdaloid complex. All the nuclei of the corticomedial and the basolateral groups are shown.

Fig. 4. Drawing of the amygdaloid nuclei near the caudal end of the amygdaloid complex.

Modified after Fox (1940).

Frontal sections. Magnification: 6X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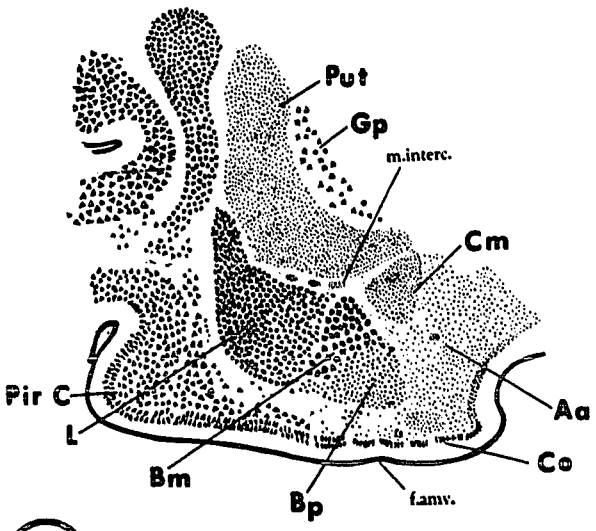
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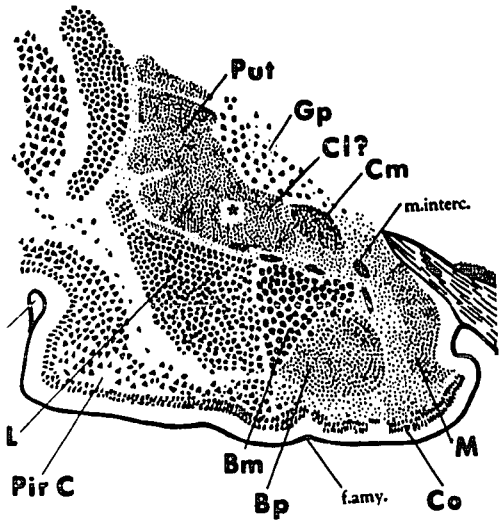
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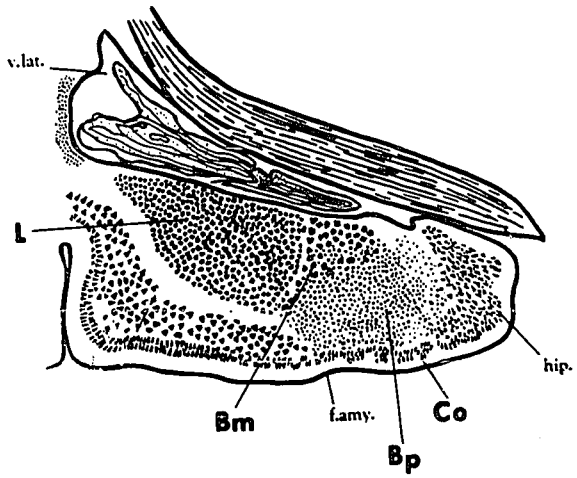
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subdivision into medial and lateral parts in man (Crosby and Humphrey, 1941), the shrew (Crosby and Humphrey, 1944), in various mammalian species described by Koikegami (1963), and in the guinea pig (Hall and Geneser-Jensen, 1971).

The medial nucleus in the cat is the largest nuclear mass of the corticomedial group (fig. 3). It is clearly recognized in the opossum (Johnston, 1923; van der Sprenkel, 1926), the bat (Humphrey, 1936), the rabbit (Young, 1936), the mink (Jeserich, 1945), the rat (Brodal, 1947), the guinea pig (Johnson, 1947a), the mole (Johnson, 1947b) and the porpoise (Breathnach and Golby, 1954), but becomes less well differentiated in man (Crosby and Humphrey, 1941).

The central nucleus in the cat (figs. 2 and 3) is the most dorsal of the amygdaloid nuclei and lies ventromedial to the putamen (Fox, 1940). It has been divided into a medial and lateral part in the cat (Fox, 1940), the mink (Jeserich, 1945) and the rat (Brodal, 1947). The medial division is easily distinguished from the lateral part by its large and deeply staining cells. The lateral division has small, lightly staining cells which are very similar to those found in the putamen. In Nissl preparations of the amygdala in the cat, the boundary between the lateral division (fig. 3 at asterisk) and the

putamen is indistinct and is marked only by a slight interruption of the cells, where the fibre bundles are passing through the inferior portion of the putamen. Koikegami (1963) has labeled the lateral part of the central nucleus the 'caudate-putamen nucleus' in his illustration of the amygdala of the cat. The portion he has labeled the lateral subdivision is represented by a small mass of cells in a position ventral to the medial part of the central nucleus.

In some species there is no large-celled portion of the central nucleus. In these, the central nucleus is a homogeneous area of small cells which are similar in appearance to those of the putamen (Humphrey, 1936 in the bat; Crosby and Humphrey, 1941 in man; Lauer, 1945 in the macaque).

Basolateral Group of Nuclei

The basal nucleus in the cat has two distinct parts, a large-celled lateral and a smaller celled medial portion (figs. 2-4). These two portions are also seen in the rabbit (Young, 1936), the bat (Humphrey, 1936), man (Crosby and Humphrey, 1941), the monkey (Lauer, 1945), the mink (Jeserich, 1945), the rat (Brodal, 1947; Yu, 1969), the porpoise (Breathnach and Golby, 1954) and the guinea pig (Johnson, 1957a). However, in the opossum (Johnston,

1923) the large-celled portion is located ventromedially and a small-celled portion is found lateral to it.

In some species the small-celled basal nucleus has been further subdivided into a deep and a superficial portion (Lauer, 1945 in the macaque; Crosby and Humphrey, 1941 in man; Breathnach and Golby, 1954 in the porpoise). An accessory basal nucleus is recognized in the opossum (Johnston, 1923; van der Sprenkel, 1926), the bat (Humphrey, 1936), the shrew (Crosby and Humphrey, 1944), the monkey (Lauer, 1945), the mink (Jeserich, 1945), the porpoise (Breathnach and Golby, 1954) and the rat (Yu, 1969) with further division into a medial and a lateral portion described in man (Crosby and Humphrey, 1941).

Although Fox (1940) did not describe an accessory basal nucleus in the cat, Karibe (1961) recognized a small mass of cells bordering the mediodorsal aspect of the large-celled basal nucleus which is similar in position to the accessory basal nucleus as described in the panda by Lauer (1949).

In all the animals investigated by Koikegami (1963) the basal nucleus showed the most complexity. This is seen especially in the ventral part of the large-celled basal nucleus which he divided into several subnuclei in such animals as the ape and man.

The lateral nucleus is a homogeneous mass in the cat (figs 2-4) as in the opossum (Johnston, 1923), the bat (Humphrey, 1936), man (Crosby and Humphrey, 1944) and the monkey (Lauer, 1945). In the rat (Gurdjian, 1928; Brodal, 1947; Yu, 1969), the rabbit (Young, 1936), the mink (Jeserich, 1945) and the guinea pig (Johnson, 1957a) two parts are described. In aquatic mammals and the monkey (Koikegami, 1963) as many as six subnuclei have been noted.

Anterior Group of Nuclei

The anterior amygdaloid area is a transitional zone of scattered cells located between the olfactory tubercle anteriorly and the amygdaloid complex posteriorly. This transitional area has been described in all mammalian species with the exception of the bat (Crosby and Humphrey, 1936).

The intercalated cell masses, also as described in all mammalian species, vary in number, size and position from animal to animal. In the cat, the intercalated nuclei are located between the basal and central and between the basal and medial amygdaloid nuclei (figs 2 and 3).

The nucleus of the lateral olfactory tract is located in the medial portion of the anterior amygdaloid area (fig. 1). In the cat (Fox, 1940) and the mink (Jeserich, 1945)

no subdivisions were noted but two parts are found in the rabbit (Young, 1936), the bat (Humphrey, 1936), the rat (Brodal, 1947; Yu, 1969), man (Crosby and Humphrey, 1941) and the shrew (Crosby and Humphrey, 1944).

Connections of the Amygdaloid Nuclei

It is not known to what extent the morphological differentiation of these nuclei reflects differences in their fibre connections. The normal fibre studies of earlier workers (Johnston, 1923; van der Sprenkel, 1926; Young, 1936; Humphrey, 1936) did not allow a determination of the direction of fibre conduction or the exact area of axonal termination. The silver techniques impregnate the products of anterograde degeneration but there is not always agreement regarding their area of termination in the amygdala of different species or even of the same species.

A) Olfactory Connections

An example of this lack of agreement is seen in the experimental studies of the termination of the lateral olfactory tract in the amygdala. A review of olfactory tract connections in mammals was presented recently by Scalia (1968). He showed that the conflicting reports concern mainly the existence of connections with the medial and central amygdaloid nuclei and the bed nucleus

of the stria terminalis. Scalia was unable to explain the failure of some investigators to find projections to these areas as due to either the techniques used or to a species difference. Only the lateral part of the cortical nucleus of the amygdala is consistently found to receive olfactory tract fibres in all the articles reviewed by Scalia.

Recently a new afferent input to the molecular layer of the medial and cortical amygdaloid nuclei has been described following a lesion in the accessory olfactory bulb in the rabbit (Winans and Scalia, 1970). According to Winans and Scalia (1970), the fibres from the accessory olfactory bulb follow the lateral olfactory tract but have not been described by investigators who used the conventional Nauta-Gygax techniques. These authors state that "The poorly myelinated segment of the accessory olfactory bulb efferents is readily detected, however, in sections of rabbit brain stained by the Fink-Heimer method."[1]

Not all mammals possess a vomeronasal organ and accessory olfactory bulb. They have not been described in the bat (Humphrey, 1936) or in adult man (Crosby et al., 1962). They were described by Fox (1940) in the cat.

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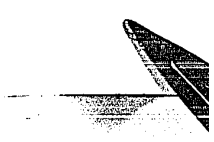
1 Winans, S. and F. Scalia, 1970, page 330.

However, in this species no mention has been made of any differences in the connections of the lateral olfactory tract which might be attributed to a lesion encroaching upon the accessory olfactory bulb (Cragg, 1961; Lohman and Lammers, 1961 and 1963; Mascitti and Ortega, 1966).

B) Cortical Connections

Since the cortical nucleus of the amygdala receives olfactory afferents and has a cortex-like layering, it has been suggested that it should be considered a part of the cortex rather than a nucleus of the amygdaloid complex (Girgis, 1965; Valverde, 1965). In addition, Valverde (1965) has noted in Golgi preparations of young cats that the cortical nucleus sends fibres to the basal and the lateral nuclei of the amygdala. This projection is similar to that from the piriform cortex which has been reported in an experimental study in the rat (Cowan et al., 1965), therefore the cortical nucleus and piriform cortex are similar in this respect.

Neocortical fibres to the lateral and basal nuclei of the amygdala have also been reported (Whitlock and Nauta, 1956; Klinger and Gloor, 1960; Showers and Lauer, 1961; Powell, 1964; Druga, 1969; Lescault, 1969, 1971). Bundles of fibres which interconnect the neocortex with the basal and lateral nuclei have been described in the normal human



brain (Klinger and Gloor, 1960). In an experimental study in the monkey, Whitlock and Nauta (1956) determined that the fibres from the temporal lobe which were afferent to the amygdala arise from the rostral part of the inferior temporal gyrus. These authors reported preterminal degeneration in the basal, lateral and central nuclei but none in the rest of the amygdala. Showers and Lauer (1961) reported fibres from the superior temporal gyrus to the basal and lateral nuclei.

There is abundant evidence of neocortical projections to the amygdala in the cat. Following selective lesions in the temporal, fronto-orbital and parietal cortices, Lescault (1969, 1971) was able to trace fibres to the amygdala from the temporal pole and the sylvian and ectosylvian gyri. These fibres course in the external capsule to terminate in the lateral nucleus and the lateral part of the central nucleus. In a similar study, Druga (1969) also observed that the sylvian gyrus projects to the amygdala. However, he was unable to find any fibres from the ectosylvian gyrus to the amygdala. Although Lescault stated that the orbital gyrus projected only to the ventromedial part of the lateral nucleus, other workers have reported fibres to the corticomedial nuclei as well (Valverde, 1965; Hirata, 1965; Mizuno et al., 1969).

However, Mizuno et al. have suggested that the fibres to the corticomedial nuclei may be the result of concomitant damage to the prepiriform cortex, olfactory tubercle or lateral olfactory tract.

The presence of terminal degeneration in the lateral nucleus after lesions of the temporal cortex has been confirmed with the electron microscope by Hall and Prym (1971). These authors stated that the degenerated boutons were in synaptic contact with dendritic spines or small dendrites. The boutons contained round vesicles and corresponded to bouton types 1 and 2 which were described in the normal amygdala (Hall, 1968).

C) Thalamic Connections

A projection from the amygdala to the mediodorsal, pulvinar and lateral posterior thalamic nuclei was first described in the monkey by Fox (1949) using the Marchi method. The amygdaloid projection to the mediodorsal thalamic nucleus was later confirmed by Nauta and Valenstein (1958) and Nauta (1961). Evidence of a reciprocal projection from the mediodorsal nucleus to the amygdala was obtained in the monkey by Nauta (1962). This author described degenerated fibres from the magnocellular division of the mediodorsal nucleus coursing in the inferior thalamic peduncle to terminate in the basal,

lateral, medial and central amygdaloid nuclei. In the cat, Valverde (1965) followed fibre degeneration from the amygdala to the caudal mediodorsal thalamic nucleus but the possibility of a reciprocal connection has not been established in this species.

Valverde (1965) reported a small amount of degeneration in the stria terminalis following a lesion of the intralaminar thalamic nuclei. Terminal degeneration in the amygdala was located in the medial division of the central nucleus.

Graybiel (1970) observed a significant projection to the caudoputamen and lateral amygdaloid nucleus following a lesion of the pulvinar - posterior complex of the thalamus in the cat.

D) Hypothalamic Connections

The stria terminalis is the most frequently described efferent pathway from the amygdala to the hypothalamus (Johnston, 1923; Gurdjian, 1925; van der Sprenkel, 1926; Young, 1936; Humphrey, 1936; Fox, 1940, 1943; Ban and Omukai, 1959; Klinger and Gloor, 1960; Hall, 1963; Leonard and Scott, 1971). Five components were noted by Johnston (1923) in the normal opossum brain. Bundles 2 (hypothalamic), 3 (infracommissural) and 4 (supracommissural) terminate in the hypothalamus. Bundle 1 (commissural)

contained fibres from the nucleus of the lateral olfactory tract to the same nucleus on the contralateral side. Bundle 5 (stria medullaris) joined the fibres of the stria medullaris to the habenular nuclei. Similar components have been described by Gurdjian (1928) in the rat, by Young (1936) in the rabbit, by Humphrey (1936) in the bat, and by Fox (1940) in the cat. However, in these species most of the hypothalamic bundle (bundle 2 of Johnston) terminates in the preoptic area and is referred to as a preoptic or postcommissural component rather than a hypothalamic component.

Gurdjian (1928), Young (1936) and Humphrey (1936) have described the stria terminalis as arising entirely from the corticomedial group of nuclei. However, Johnston (1923) and van der Sprenkel (1926) reported that fibres from the basal and lateral nuclei also joined the stria terminalis. In experimental studies in the cat (Fox, 1943; Hall, 1963), the rabbit (Ban and Omukai, 1959) and the rat (Leonard and Scott, 1971) it was confirmed that some fibres from the basal nucleus enter the stria terminalis but those from the lateral nucleus do not (Fox, 1943; Ban and Omukai, 1959; Hall, 1963).

The experiments of Nauta (1961) in the monkey suggested that only the caudal half of the amygdala projected through the stria terminalis. These observations

were not supported by Valverde (1965) in his studies of the cat. This author found that although the commissural component of the stria terminalis originated from the caudal part of the amygdala, the supracommissural and postcommissural components originated from the rostral part.

Because of their small calibre, it is extremely difficult to follow the fibres of the stria terminalis to their nuclei of termination (Fernandez de Molina and Garcia-Sanchez, 1967; Heimer and Nauta, 1969). Therefore, the experimental studies have shown conflicting results.

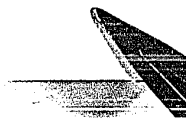
Fox (1940), using the Marchi method in the cat, noted that the stria terminalis projected only to the rostral half of the hypothalamus. This observation was reinforced by studies in the monkey (Nauta, 1961), the cat (Szentagothai et al., 1962) and the rat (Cowan et al., 1965) with the Nauta-Gygax method. However, in the monkey (Adey and Myer, 1952) and the rabbit (Ban and Omukai, 1959) some fibres of the stria terminalis were reported to project as far caudally in the hypothalamus as the ventromedial nucleus. This projection to the ventromedial hypothalamic nucleus was confirmed in the cat by Hall (1963). Even more recently, in a study in the rat using the Fink and Heimer method, the supracommissural fibres of the stria terminalis have been followed as far caudally as the

ventral premammillary nuclei (Heimer and Nauta, 1969).

The amygdala also contributes fibres to the hypothalamus via a shorter ventral route, the longitudinal association bundle. This bundle first appears in marsupials and gains importance in higher mammals as the main efferent pathway of the basal and lateral amygdaloid nuclei (Johnston, 1923). Fox described the longitudinal association bundle in both normal (1940) and experimental (1943) studies in the cat. He stated that fibres from the piriform lobe and lateral amygdaloid nucleus form a bundle which coursed dorsomedially and rostrally through the amygdaloid complex. As this bundle continued rostrally it increased in size by the addition of fibres from the anterior part of the basal nucleus. Near the anterior end of the basal nucleus it curved dorsally through the central nucleus, where it split into a posterior and an anterior bundle. The posterior one coursed medialward to the preoptic area, while the anterior portion continued rostrally through the anterior amygdaloid area before turning medialward to end in the bed nucleus of the stria terminalis. Hall (1963) confirmed the finding of Fox that part of this ventral pathway originates in the basal and lateral nuclei. In addition, she stated that some fibres from the lateral nucleus

course directly medialward between the ansa lenticularis and optic tract to terminate in the preoptic area and throughout the lateral hypothalamus. Because of the separate course of the medially directed pathway, Valverde (1965) referred to it as the medial hypothalamic pathway rather than as a portion of the longitudinal association bundle. In agreement with Hall, he found that the medial pathway terminated throughout the lateral hypothalamus but he reported a more extensive distribution of fibres from the rostrally coursing longitudinal association bundle. The latter terminated in the lateral preoptic area and anterior limbic region. This more extensive distribution of the longitudinal association bundle had been previously described in experimental studies of the monkey by Nauta and Valenstein (1958) and Nauta (1961). Nauta (1961) introduced the term ventral amygdalofugal pathway in reference to the entire ventral amygdalo-subcortical fibre system.

It is important to note that Powell et al., (1963) described degenerated fibres of the longitudinal association bundle after piriform cortex lesions that have the same course and distribution as those reported following lesions of the amygdala. These authors stress the fact that any lesion of the amygdala interrupts these fibres



from the piriform cortex so that the contribution of the amygdaloid nuclei to the ventral pathway is difficult to determine.

It is generally accepted that there are two main efferent pathways from the amygdala, the stria terminalis and a more diffuse ventral amygdalofugal pathway. It has not been generally recognized, however, that both of these pathways contain amygdalopetal fibres.

Bucher and Bürgi (1953), using the Marchi method in the cat, reported the presence of amygdalopetal fibres in the stria terminalis following a lesion of the caudal hypothalamus but they were unable to follow the fibres to their termination. In an investigation of the limbic pathways in the cat, Nauta (1958) observed afferent fibres in both the stria terminalis and the ventral pathway, after placing a lesion in the lateral preoptic area. However, the lateral hypothalamic area contains the medial forebrain bundle and Nauta pointed out that a lesion of the lateral preoptic area necessarily interrupted fibres of passage in the medial forebrain bundle so that some of the amygdalopetal fibres might originate from more caudal hypothalamic areas. In recognition of this fact, Cowan et al. (1965) made a series of lesions in the rat which involve the lateral preoptic area and various hypo-

thalamic areas caudal to it. They confirmed the observations of Nauta concerning afferent fibres in the stria terminalis and demonstrated that the ventral pathway, although more diffuse, is the larger amygdalopetal pathway from the hypothalamus. Only the rostral hypothalamus was found to contribute afferent fibres to the stria terminalis and the ventral pathway. Terminals were observed in the basal and medial nuclei in the rat (Cowan et al., 1965) as in the cat (Nauta, 1958). Although terminal degeneration was noted in the posterior part of the lateral nucleus in the rat, no degeneration was seen in the lateral nucleus in the cat. Nauta observed terminal degeneration in the central nucleus of the cat, but Cowan et al. (1965) were unable to determine if these fibres terminated or passed through the central nucleus.

It can be seen from the above review that only two experimental anatomical studies have dealt with hypothalamic afferents to the amygdala and these have shown conflicting results concerning the areas of termination. This difference in results may be attributable to a species difference.

There are two other reports which suggest that the stria terminalis contains fibres afferent to the amygdala. The first of these is a histochemical study of choline-

sterase-containing systems in the rat brain. Shute and Lewis (1961) observed that sectioning of cholinesterase-containing axons causes the enzyme to disappear on the distal side of the axon. When they transected the stria terminalis they observed that the cholinesterase disappeared in the lateral portion of the stria terminalis caudal to the transection. From these observations, Shute and Lewis concluded that these axons containing cholinesterase were directed toward the amygdala. They suggested on the basis of observations in their normal material stained for cholinesterase that these axons originated in either the bed nucleus of the stria terminalis (Shute and Lewis, 1961) or the lateral preoptic area (Shute and Lewis, 1963).

The second report is an investigation of the defence reaction in cats. Stimulation of the stria terminalis elicits a pattern of behavioral responses associated with the defence reaction, such as, pupillary dilatation, pricking of the ears, piloerection on the back and tail, growling, hissing, running and urinating (Fernandez de Molina and Hunsperger, 1959; Zbrozyna, 1960). Fernandez de Molina and Hunsperger (1962) abolished this defence reaction by placing a lesion in the bed nucleus of the stria terminalis. The same result was obtained

by Zbrozyna (1963) immediately after the lesion was made. However, when the stria terminalis was stimulated several days after a transection of the stria terminalis, the defence reaction could still be elicited by stimulation of the fibres proximal to the amygdala. It could be abolished only by placing a lesion in the large-celled basal nucleus or the ventral pathway (Hilton and Zbrozyna, 1963). Zbrozyna (1963) concluded that the stria terminalis is, therefore, the afferent pathway for the defence reaction elicited from the amygdala rather than the efferent pathway.

Problem Formulation

In the review of the amygdaloid nuclei in various mammalian species, it was noted that two subdivisions of the central nucleus were identified in the cat by Fox (1940). This author described a small-celled portion which was situated lateral to a medial, large-celled portion. However, Koikegami (1963) labeled the group of small cells the 'caudate-putamen nucleus' in his illustration of the amygdala in the cat. He located the lateral subdivision of the central nucleus in a position ventral to the large-celled part. Since these two authors disagree on the location of the lateral subdivision of the central nucleus, the amygdaloid nuclei will be examined in a series of Nissl stained sections cut in either the frontal, horizontal or parasagittal plane of section. The electron microscope, as well as Nissl preparations, will be used in studying the subdivisions of the central nucleus.

An electron microscopic investigation of the medial and lateral amygdaloid nuclei (Hall, 1968) and the cortical nucleus (Hall and Prym, 1972) has already been carried out. Therefore, it will be possible to compare the fine structure of the two subdivisions of the central nucleus with the medial, lateral and cortical

amygdaloid nuclei.

Because of the similarity of the neurons in the lateral subdivision of the central nucleus and the putamen, there is difficulty in determining the border between the two nuclei in cell stains although fibre bundles have been used as a demarcation by some authors (Johnston, 1923; Fox, 1940). Even though the cells appear the same in material stained for Nissl, there may be some distinguishing features that can be seen with the electron microscope. Therefore, the putamen will be included in the electron microscopic studies.

The existence of afferent fibres in the stria terminalis to the amygdala has been demonstrated in experimental anatomical, histochemical and behavioural studies. In addition, recent anatomical studies have revealed the presence of amygdalopetal fibres in the ventral pathway as well. The review of the literature indicates that the exact origin of these fibres and their termination within the various amygdaloid nuclei remains unsettled.

It would be especially interesting to determine whether or not the ventromedial hypothalamic nucleus and the ventral premammillary hypothalamic nucleus project to the amygdala. There is evidence that these nuclei

receive amygdalofugal fibres by way of the supra-commissural portion of the stria terminalis. The possibility of a reciprocal relationship between these hypothalamic nuclei and the amygdala needs investigation.

The results of experimental anatomical investigations suggest that the lateral preoptic area is the source of the hypothalamo-amygdaloid fibres. It has been noted that the medial forebrain bundle courses through the entire anteroposterior extent of the lateral hypothalamus and would be interrupted by a lesion in this region. The possibility exists that more caudal hypothalamic areas project to the amygdala via the medial forebrain bundle.

In order to determine the origin of these amygdalopetal fibres from the hypothalamus, a series of lesions will be placed at various rostro-caudal levels of the hypothalamus of the cat. Following an appropriate survival time, the products of Wallerian degeneration will be stained by one of the modifications of the Nauta method. Since the more recent modifications of the original Nauta method are thought to impregnate preterminal and terminal degeneration more reliably, the Nauta-Laidlaw (1957), Fink and Heimer (1967) and Wiitanen (1969) methods will be used.

The use of electron microscopic techniques in this study will allow a more detailed anatomical description of the degenerated synaptic boutons and their postsynaptic sites. Electron microscopic studies of the cortical afferents to the amygdala indicate that the terminal boutons contain round vesicles and terminate on dendritic spines. It may be possible to distinguish the hypothalamic boutons by their morphological characteristics.

The electron microscopic study of the normal central nucleus will provide a control for the experimental electron microscopic study of the hypothalamic afferents to the amygdala.

CHAPTER II

Materials and Methods

The present study is based on observations made on a total of 29 cat brains subdivided as follows:

Normal Material	
Light microscopy	5 cats
Electron microscopy	4 cats

Experimental Material	
Light microscopy	15 cats
Electron microscopy	5 cats

Normal Material

A) Light microscopy

Four normal cat brains from the collection of the Department of Anatomy, University of Ottawa, were used in the study of the normal amygdala. The following slides were examined:

Series SL slides 57 to 74 -- This brain was cut in the frontal plane at a thickness of 10 microns and stained with cresyl echt violet for cells. Every tenth section was stained for fibres by the Heidenhain method.

Series 17E slides 1 to 10 -- This brain was cut in the frontal plane at a thickness of 20 microns and stained with toluidine blue for cells.

Series A slides 31 to 75 -- This brain was cut in the parasagittal plane at a thickness of 20 microns and stained with toluidine blue. Every tenth section was stained by the Heidenhain method.

Series B slides 6 to 37 -- This brain was cut in the horizontal plane at a thickness of 20 microns and stained with toluidine blue.

One brain was cut in the frontal plane at a thickness of 30 microns. Following every fifth section cut at 30 microns, one section was cut at a thickness of 90 microns. All the sections through the amygdaloid complex were stained with cresyl echt violet and the 90 micron thick sections photographed.

Light micrographs were taken with the Zeiss Ultraphot on 9 x 12 cm Kodak Ektapan sheet film.

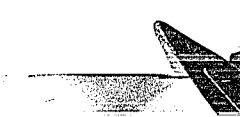
B) Electron microscopic methods

1) Anesthesia. - Healthy adult cats ranging from 2.4 to 3.5 kgs in weight were anesthetized with

an intraperitoneal injection of 50 mgs sodium nembutal per kilogram of body weight.

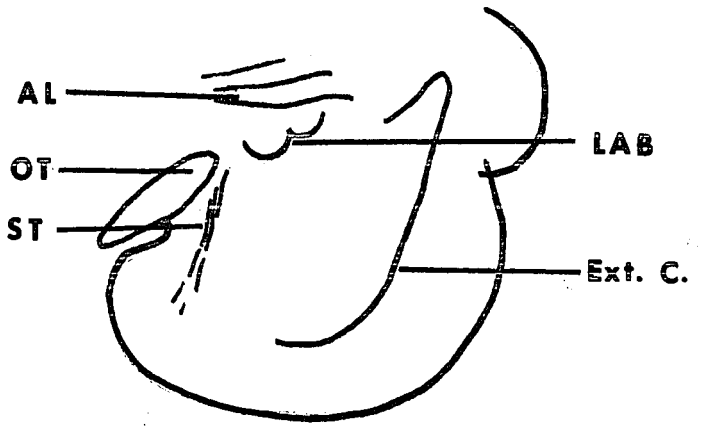
2) Perfusion. - The chest cavity was opened and the descending aorta clamped with a haemostat. The right auricle of the heart was opened and a 15 gauge hypodermic needle was inserted into the left ventricle. A total volume of 300 to 350 cc of Ringer's solution at 37°C was allowed to flow into the ventricle from a height of 120 cm above the animal's head. This was followed by 400 to 500 cc of phosphate buffered formalin at 37°C (according to Holt and Hicks, 1961). The osmolarity of the phosphate buffer was measured by freezing point depression at 340 mOs. The brain was quickly removed and allowed to fix further in the perfusate for one hour.

3) Dissection. - Frontal slices 2 to 3 mm thick were cut at the level of the amygdala and replaced into the formalin solution. The bottom of a small petri dish was covered with a thin layer of wax and about 10 cc of the fixative. A slice from the middle of the amygdala was then placed in the petri dish and observed under the dissecting



microscope.

In the freshly fixed brain tissue the fibres appeared very white against a background of gray cellular areas. Five prominent fibre bundles could be seen in the area of the amygdaloid nuclei. These fibre tracts are illustrated in the diagram below:



It can be seen from this diagram that the areas containing the five major nuclei of the amygdala are outlined by these pathways. The medial nucleus lies between the prominent optic tract and the stria terminalis. The central nucleus is located inferior to the ansa lenticularis and superior to the longitudinal association bundle. The fibres of the longitudinal association bundle also separate the medial and lateral subdivisions

of the central nucleus. The basal nucleus lies inferior to the longitudinal association bundle and lateral to the stria terminalis. The two subdivisions of the basal nucleus could not be determined with the dissecting microscope. The external capsule borders the lateral nucleus on its lateral and ventral aspects. The cortical nucleus lies just below the pial surface ventral to the area of the basal nucleus.

Blocks of tissue less than 1 mm thick were removed from each of these nuclei.

4) Post-Fixation. - The small pieces of tissue were placed in cold phosphate buffered 1% osmium tetroxide and maintained at 4°C for 1 to 2 hours.

5) Dehydration. - After post-fixation the blocks were rinsed in Ringer's solution for 15 minutes and then dehydrated in a graded series of 30%, 50%, 75%, 90% and 100% acetone.

6) Embedding. - Each block of tissue was placed successively into a 3:1, 1:1 and 1:3 series of acetone/vestopal mixtures. They were left in each of these mixtures for two hours on a rotator. They were then put in 100% vestopal and placed on a rotator overnight. Each piece of tissue was embedded in a size 00 gelatine capsule

containing vestopal with 1% initiator and 1% activator. The vestopal was polymerized at 40°C for 24 hours and then at 60°C for 48 hours.

7) Sectioning. - Thin sections showing an interference color of silver to gray were cut from blocks of the central nucleus and collected on a copper grid covered with a thin film of parlodion.

8) Staining. - The sections were stained in 1% lead citrate (according to Reynolds, 1963) for three to five minutes.

9) Microscopy. - A Zeiss 9A electron microscope was used for viewing.

Experimental Material

A) Light microscopic methods

1) Operative procedures. - Healthy adult cats ranging in weight between 2.5 and 3.5 kgs were anesthetized with 50 mg/kg body weight of sodium nembutal. Each cat was also given 1 cc of penicillin G by intramuscular injection. The head of the animal was fixed in a Kopf stereotaxic instrument. Lesions were placed by means of stainless steel electrodes inserted through either the contralateral hemisphere or the rostral cortex of the same hemisphere (see Tables I - III). A Wyss high frequency coagulator produced a round, well localized lesion of approximately 1 mm in diameter when set at 4 ma for 30 seconds. Fifteen of the cats had satisfactory lesions (Tables I - III).

2) Survival period. - The animals were sacrificed 4, 8 or 11 days after the lesion had been made.

3) Perfusion. - The cats were anesthetized with sodium nembutal and perfused through the heart with 250 cc of physiological saline followed by 350-400 cc of 10% formalin. The brain was removed and placed in 10% formalin for at least

Lesions of the hypothalamus at the tuberal level

Lesion	Cat Number	Survival Period	Technique	Plane of Section	Electrode Approach	Extent of Lesion
Ventro-medial Hypo-thalamic Nucleus	HW 18	4 days	Fink and Heimer	Frontal	Contralateral	This lesion was limited to the rostral 2/3rds of the ventro-medial nucleus of the hypo-thalamus.
	HW 29	8 days	Nauta-Laidlaw Fink and Heimer	Frontal	Contralateral	The lesion involved the posterior 2/3rds of the ventromedial nucleus and extended into the rostral portion of the posterior hypo-thalamic nucleus.
Lateral Hypo-thalamic Area	HW 28	8 days	Nauta-Laidlaw	Frontal	Contralateral	This small lesion was in the medial forebrain bundle at the level of the ventromedial nucleus.
	HW 30	4 days	Wiitanen	Frontal	Contralateral	This was a small lesion limited to the lateral hypo-thalamic area at the level of the tuber cinereum.
	HW 26	8 days	Fink and Heimer Nauta-Laidlaw	Frontal	Contralateral	The lesion inter-rupted the medial forebrain at the level of the tuber cinereum. It also involved the lateral part of the ventro-medial nucleus.

Lesions of the anterior hypothalamus

Lesion	Cat Number	Survival Period	Technique	Plane of Section	Electrode Approach	Extent of Lesion
Anterior Hypothalamic Nucleus	HW 25	11 days	Nauta-Laidlaw	Frontal	Contralateral	The lesion involved the anterior hypothalamic nucleus at its rostral extreme. It extended to the suprachiasmic nucleus, area of the tuber cinereum and slightly into the lateral hypothalamic area at the level of the tuber cinereum.
Medial Preoptic Area	CA 5	11 days	Nauta-Laidlaw Fink and Heimer	Frontal	Ipsilateral through rostral cortex	The lesion was localized within the medial and superior preoptic area and involved a small part of the bed nucleus of the anterior commissure.
	HW 32	8 days	Nauta-Laidlaw Wiitanen	Frontal	Contralateral	This was a small lesion which involved the inferior portion of the medial preoptic area.
	HW 19	11 days	Nauta-Laidlaw	Horizontal	Contralateral through rostral cortex	This was a small lesion localized within the medial preoptic area.

Lesions of the anterior hypothalamus (lateral preoptic area)

Lesion	Cat Number	Survival Period	Technique	Plane of Section	Electrode Approach	Extent of Lesion
Lateral Preoptic Area	HW 1	4 days	Fink and Heimer	Frontal	Contralateral	The area of coagulation was limited to the caudal part of the lateral preoptic area.
	HW 2	8 days	Nauta-Laidlaw Fink and Heimer	Frontal	Contralateral	Same as the above.
	HW 3	8 days	Nauta-Laidlaw	Frontal	Contralateral	Same as the above.
	HW 4	8 days	Nauta-Laidlaw	Sagittal	Contralateral	Same as the above.
	HW 5	8 days	Nauta-Laidlaw	Frontal	Ipsilateral through rostral cortex	The area of damage was localized mainly in the superior part of the lateral preoptic areas and extended slightly into the bed nucleus of the anterior commissure and the anterior hypothalamic area.
	HW 6	8 days	Nauta-Laidlaw Fink and Heimer	Frontal	Ipsilateral through rostral cortex	Same as cat HW 5 but with less involvement of the anterior hypothalamic area.

6 weeks prior to staining.

4) Sectioning. - The brain was frozen with dry ice and sectioned on a sliding microtome at a thickness of 30 microns. When the level of the lesion was reached, sections 90 microns thick were cut alternately with five sections at 30 microns.

5) Staining. - Every tenth section (30 microns thick) was stained with the Nauta-Laidlaw (1957), the Fink and Heimer (1967) or the Wiitanen (1969) method for the demonstration of degenerated axons. Corresponding sections were stained for cells with cresyl echt violet to allow verification of the specific nuclei of termination.

6) Drawings. - Every tenth section, stained with cresyl echt violet, was placed in a photographic enlarger and projected onto drawing paper. The outlines of the nuclei were traced onto the paper. When necessary, the fibre tracts were traced from corresponding sections stained by the Nauta method. The sections stained for degenerated axons were scanned with the light microscope and the course and distribution of the degeneration marked on the drawings.

7) Photography. - Light micrographs of the degeneration were taken with the Zeiss Ultraphot on 9 x 12 cm Kodak Ektapan sheet film. The thick sections were photographed according to the method of Guzman et al. (1958). As described in this method, each section was mounted from 50% alcohol onto a glass slide and placed in a photographic enlarger in the same manner as a negative. The image was then printed on F5 photographic paper. The section was allowed to dry and then stained with cresyl echt violet to check the full extent of the lesion.

B) Electron microscopic methods

1) Operative procedures. - The animal was anesthetized and placed in the stereotaxic apparatus as described for the experimental animals in the light microscopic section. A lesion was placed in the lateral preoptic area by means of an electrode inserted through the contralateral hemisphere. Five of the cats had a large lesion of the lateral preoptic area (Table IV).

2) Survival period. - The animals were allowed to survive for a period of 2, 3, 5, 7 or 15 days.

3) Perfusion. - The animals were anesthetized with sodium nembutal and perfused through the heart with 300-350 cc of Ringer's solution at 37°C followed by 400-500 cc of phosphate buffered formalin at 37°C as described in the electron microscopic methods of the normal material.

4) Dissection. - Blocks of tissue less than 1 mm thick were removed from all of the amygdaloid nuclei in the same manner as in the normal electron microscopic study.

Lesions of the Lateral Preoptic Area (prepared for Electron Microscope)

Cat No.	Survival Period	Extent of the Lesion	Nuclei Viewed with the Electron Microscope						
			Cl	Cm	M	Bm	Bp	L	Co
EEM 1	2 days	This lesion was limited to the caudal ½ of the lateral preoptic area.	X			X			0
EEM 2	3 days	This lesion was limited to caudal 2/3rds of lateral preoptic area.				X	X	X	
EEM 3	5 days	This lesion involved the entire antero-posterior extent of the lateral preoptic area. The lesion also interrupted the inferior portion of the anterior commissure and the rostro ventral portion of the globus pallidus.	X	X	X	X	X	X	0
EEM 4	7 days	Large lesion of the lateral preoptic area which extended slightly into the medial preoptic area.	X		X	X			0
EEM 5	15 days	This lesion was limited to the caudal 2/3rds of the lateral preoptic area.	X	X			X	X	0

X -- nuclei viewed - degeneration present

0 -- nuclei viewed - no degeneration present

5) to 7) Post-Fixation, dehydration and embedding methods were exactly the same as in the normal electron microscopic study.

8) Sectioning. - Thin sections were cut from blocks of each nucleus of the amygdala. Several nuclei were examined from each survival period and are listed in table IV. Semithin sections were cut from blocks of the basal nucleus to determine which subdivision it was taken from.

9) Staining. - The thin sections were stained in 1% lead citrate. The semithin sections were stained with toluidine blue.

10) Microscopy. - The thin sections were viewed with a Zeiss 9A electron microscope. The semithin sections were viewed on a Zeiss Ultraphot.

11) Verification of lesion. - After the blocks had been removed from the amygdala, the remaining tissue was placed in 10% formalin for 6 weeks. The slices were then dehydrated and embedded in celloidin. They were sectioned at a thickness of 30 microns and stained by the thionin method for Nissl substance. The

sections were then examined with the light microscope to determine the extent of the lesion in the preoptic area. The limits of each lesion are described in table IV.

Abbreviations

A	astrocyte
Aa	anterior amygdaloid area
Acb	nucleus accumbens
AL	ansa lenticularis
B	bouton
B_1	bouton with round vesicles
B_2	bouton with round vesicles of varied diameter
B_3	bouton with flattened vesicles
B_4	bouton with dark core vesicles
Bm	magnocellular division of the basal amygdaloid nucleus
Bp	parvocellular division of the basal amygdaloid nucleus
C	subsurface cistern
CA	anterior commissure
Cd	caudate nucleus
CI	internal capsule
CL	lateral division of the central amygdaloid nucleus
Cm	medial division of the central amygdaloid nucleus
Co	cortical nucleus of the amygdala
D	dendrite

Abbreviations (continued)

dA	degenerated axon
dB	degenerated bouton
DBB	diagonal band of Broca
En	entopeduncular nucleus
Ext. c.	external capsule
f. amy.	amygdaloid fissure
G	golgi apparatus
Gp	globus pallidus
hip	hippocampus
Hp	posterior nucleus of the hypothalamus
Interc.	intercalated nucleus
L	lateral amygdaloid nucleus
L (EM)	lysosome
LAB	longitudinal association bundle
LD	nucleus lateralis dorsalis of the thalamus
LP	nucleus lateralis posterior of the thalamus
LPO	lateral preoptic area
M	medial nucleus of the amygdala
M (EM)	mitochondrion
MD	nucleus medialis dorsalis of the thalamus
MV	multivesicular body
nhvm	ventromedial nucleus of the hypothalamus
Nlot	nucleus of the lateral olfactory tract

Abbreviations (continued)

NUC	nucleus
O	oligodendrocyte
OT	optic tract
Pir. C.	piriform cortex
Put	putamen
RE	nucleus reuniens of the thalamus
S	soma
SA	spine apparatus
Sp	spine
Spl	lateral nucleus of the septum
SSp	soma spine
ST	stria terminalis
v. lat.	lateral ventricle
VA	nucleus ventralis anterior of the thalamus
VL	nucleus ventralis lateralis of the thalamus
VM	nucleus ventralis medialis of the thalamus
VP	nucleus ventralis posterior of the thalamus

CHAPTER III

Observations

Normal Material

A) Light microscopy

The basic description of the amygdala in the cat as presented by Fox (1940) is very complete. However, a few observations can be added.

Corticomedial group of nuclei:

In the cat, a large-celled portion of the central nucleus is distinguished from a smaller celled more lateral part (figs 5 to 8). The cells in the lateral subdivision are similar in size, shape and staining to the small cells of the putamen. Because of this similarity in cell type between the lateral division of the central nucleus and the putamen, Fox (1940) has suggested that the lateral division be designated as a putamen-central amygdaloid complex. However, the present material indicates the presence of two types of cell in the putamen (figs 6 and 7). The small, lightly staining cells are similar to those in the lateral division of the central nucleus. But it is noted that the putamen contains a number of large, deeply stained cells scattered throughout the nucleus

Fig. 5. Light micrograph of the rostral part of the amygdaloid complex.

Fig. 6. Light micrograph of the central part of the amygdaloid complex.

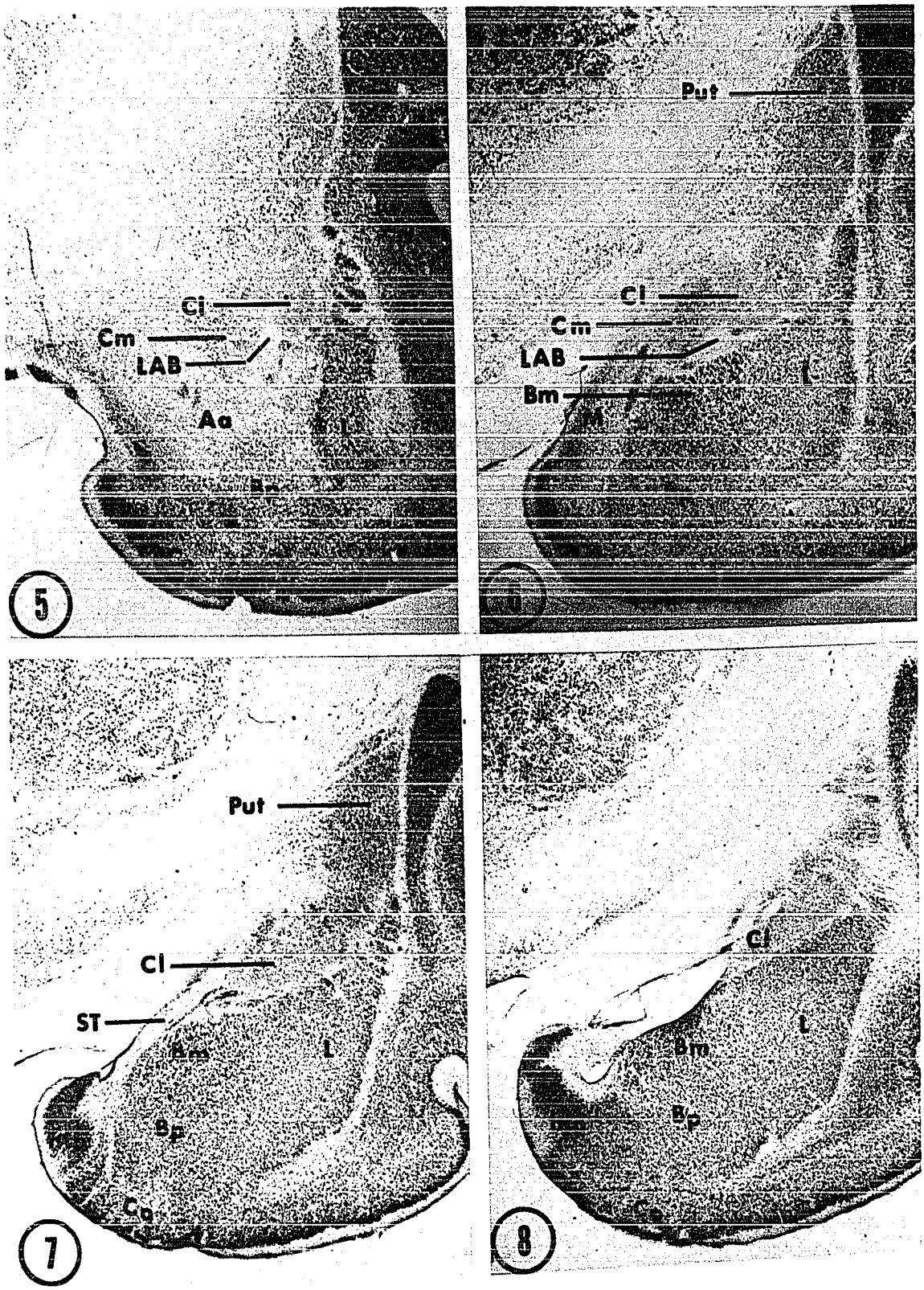
Fig. 7. Light micrograph of the central part of the amygdaloid complex at the level of the stria terminalis.

Fig. 8. Light micrograph of the caudal amygdaloid complex.

Frontal plane of section.

Cresyl echt violet stain.

Magnification: 9X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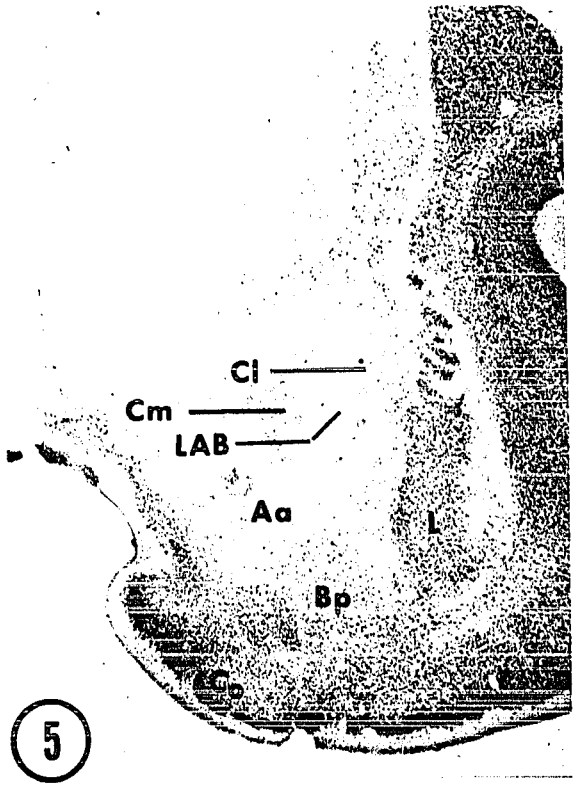
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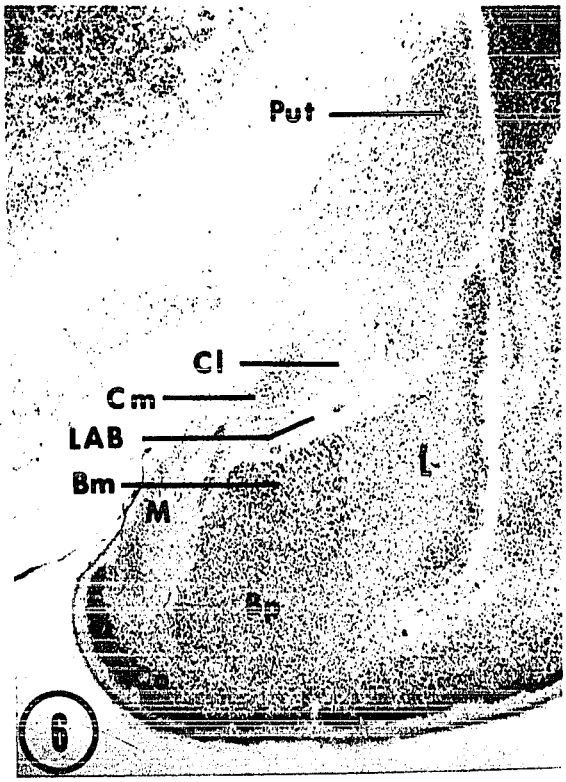
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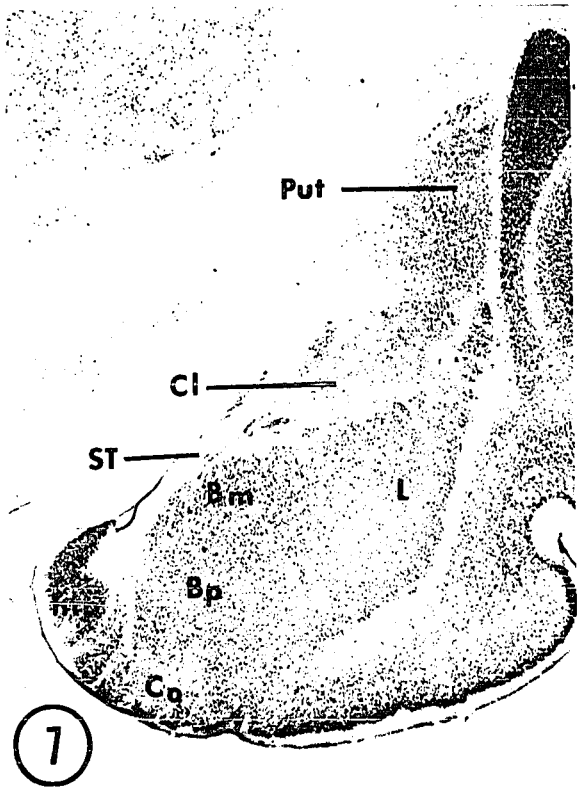
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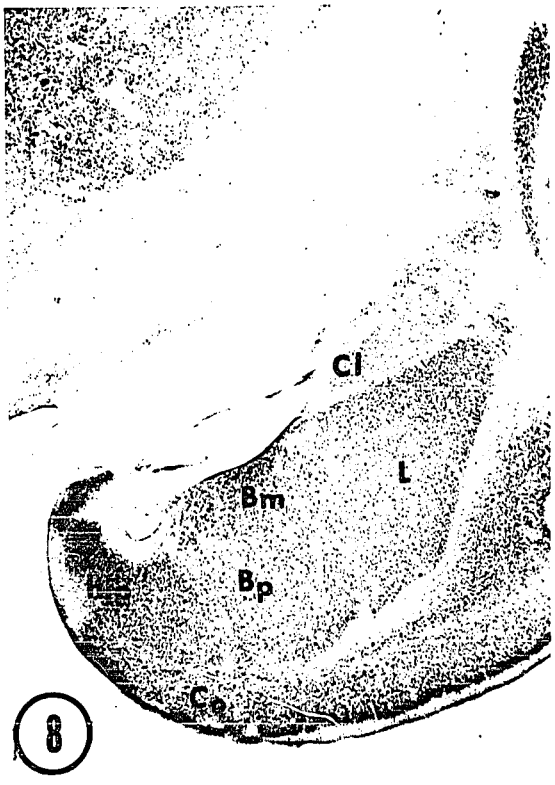
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but the lateral division of the central nucleus contains only small cells. Therefore, the lateral division of the central nucleus may be identified by its homogeneous population of small cells that distinguish it from the putamen, which contains two cell types; and from the medial division of the central nucleus, which contains a homogeneous population of large, deeply stained cells.

The medial nucleus contains medium sized cells that stain lightly (fig. 6). Fibres of the stria terminalis course through the caudal portion. Rostrally the medial nucleus merges with the anterior amygdaloid area.

The cortical nucleus is the most ventral of the amygdaloid nuclei. Pyramidal shaped cells form a distinct layer that is more loosely arranged in the lateral half than in the medial half (figs 5 and 6). The boundary between the piriform cortex and the cortical nucleus is marked by the amygdaloid fissure. No cortico-amygdaloid transitional zone was identified in this material. The cells in the deeper portion of the cortical nucleus are similar to those of the small-celled basal nucleus. There is no clear boundary between these two nuclei (figs 7 and 8).

Basolateral group of nuclei:

The lateral nucleus (figs 5 to 8) contains a homogeneous population of large cells. The dorsolateral portion of the lateral nucleus is traversed by small bundles of fibres and has been considered by some investigators to form a separate division (Koikegami, 1963).

The two divisions of the basal nucleus are easily identified (figs 6 to 8). The cells in the large-celled portion are the largest and most deeply staining cells in the amygdaloid complex. Karibe (1961) identified a small group of cells bordering the dorsomedial side of the large-celled basal nucleus as an accessory basal nucleus. This group of cells could not be identified in this material.

Anterior group of nuclei:

The scattered cells of the anterior amygdaloid area can be seen in figure 5. The most rostral parts of the lateral, central and small-celled basal nucleus are also present.

The intercalated masses are seen as small, closely grouped neurons located ventral to the central nucleus and dorsal to the large-celled part of the basal nucleus. These intercalated neurons can be seen in

figure 6 where they form two very small and deeply staining cellular areas, one on each side of the longitudinal association bundle.

The nucleus of the lateral olfactory tract is situated in the medial portion of the anterior amygdaloid area. It appears as a small spherical mass of deeply staining cells and has no subdivisions.

B) Electron microscopic observations of the central nucleus

The perikarya in the two divisions of the central nucleus are very similar in appearance (compare figs 9 and 16). The soma of each neuron is occupied by a large nucleus which contains a nucleolus and scattered chromatin material in the pale staining karyoplasm. The cytoplasm surrounding the nucleus contains many ribosomes clustered in the form of rosettes or attached to the endoplasmic reticulum. The granular endoplasmic reticulum is arranged as isolated cisterns or in stacks of two or three short cisterns. Occasionally a subsurface cistern is seen close to the plasma membrane of the cell body (fig. 15). The end of the cistern is dilated and its deep surface has ribosomes attached to it. Prominent in the cytoplasm are multivesicular bodies, lysosomes, granular vesicles, mitochondria and elements of the Golgi apparatus.

The membrane of the nucleus is very irregular and has two or three invaginations which contain many ribosomes. Some of these ribosomes are attached to the cytoplasmic surface of the nuclear envelope. The interspace between the double membrane of the nuclear envelope is in communication with cisterns of the endo-

plasmic reticulum. The somatic membrane also has very irregular contours with an occasional spine-like projection in contact with a synaptic bouton (fig. 18). The somatic membrane is in contact with glial cells or their processes, boutons, small axons and dendrites.

The origin of the axon from the axon hillock is recognized by the presence of a dense material beneath the plasma membrane of the initial segment as described by Palay et al. (1968). The neurotubules in the initial segment appear in groups rather than as single elements as in the more distal portion of the axon. The axon contains mitochondria, agranular endoplasmic reticulum, multivesicular bodies, neurofilaments and neurotubules. It does not contain ribosomes or granular endoplasmic reticulum.

The cytoplasm of the dendritic process contains the same organelles as the cytoplasm of the soma; that is, multivesicular bodies, mitochondria, endoplasmic reticulum, granular vesicles and ribosomes. Also present are a few neurofilaments and numerous neurotubules both of which are arranged parallel to the length of the dendrite. In many cases a spine could be seen branching from the dendritic trunk. The cytoplasm of the spine contains a fluffy material that consists of fine filaments as has been described previously by Peters et al. (1970). The

cytoplasm of the spine occasionally contains a spine apparatus which consists of a series of sacs separated by plaques of dense material. The spine apparatus is not an exclusive feature of dendritic spines since it has also been described in dendritic trunks (Westrum and Blackstad, 1962; Hall, 1968). However it was not seen in any of the dendrites of the central nucleus.

Many synaptic contacts are seen along the surface of the dendrites, their spines, and, more rarely, the cell soma. The areas of synaptic contact are identified by the thickening and increased density of the apposed pre- and postsynaptic membranes and an increase in the intercellular space between the two specialized membranes (figs 10-12, 14a, 17-19). In some synapses the post-synaptic membrane is bordered on the cytoplasmic side by an accumulation of dense material (figs 10, 11, 17, 18). In rare instances, a row of dense round bodies is present in the postsynaptic cytoplasm parallel to the postsynaptic opacity (figs 14a and b). Associated with the areas of membrane specialization on the presynaptic side are aggregations of synaptic vesicles. The pre-synaptic profiles contain, in addition to the vesicles, mitochondria and multivesicular bodies. Profiles such as these were sometimes seen to emerge from a myelin

sheath and were interpreted as the terminal boutons of axons.

Most of the terminal boutons within both subdivisions of the central nucleus can be assigned, on the basis of the size and shape of their vesicles, to four groups as described in other amygdaloid nuclei by Hall (1968).

The first group consists of boutons which contain round synaptic vesicles of a uniform diameter. Examples of these boutons are shown in figures 10 and 17 (B_1). The postsynaptic profiles are identified as dendrites by the presence of ribosomes in the cytoplasm. Note the presence of a definite postsynaptic density bordering the postsynaptic membrane. The bouton shown in figure 15 also belongs to this group because of the uniform diameter of the vesicles. However, the postsynaptic density is not as obvious in some axosomatic contacts.

Boutons belonging to the second group are recognized as a separate type because the vesicles are of varying diameter. Examples of these boutons are shown in figures 11 and 18. Note that the synaptic contact in figure 18 is with a soma spine.

The boutons included in groups one and two are most frequently found in contact with dendrites or

their spines but are occasionally in contact with a cell soma.

Boutons assigned to the third group are the most common type observed in the medial and lateral subdivisions of the central nucleus. This bouton is characterized by the presence of predominately flattened vesicles (B_3; figs 12 and 19). Note the absence of the dense material in the postsynaptic cytoplasm. Terminals containing flattened vesicles are found in synaptic contact with dendrites or cell somata but never with dendritic spines.

The axon terminals of the fourth group are very rarely seen in the central nucleus. These terminals contain many dark core vesicles (B_4 in figures 13 and 20) and seldom are observed to form synaptic contacts.

Glial Cells

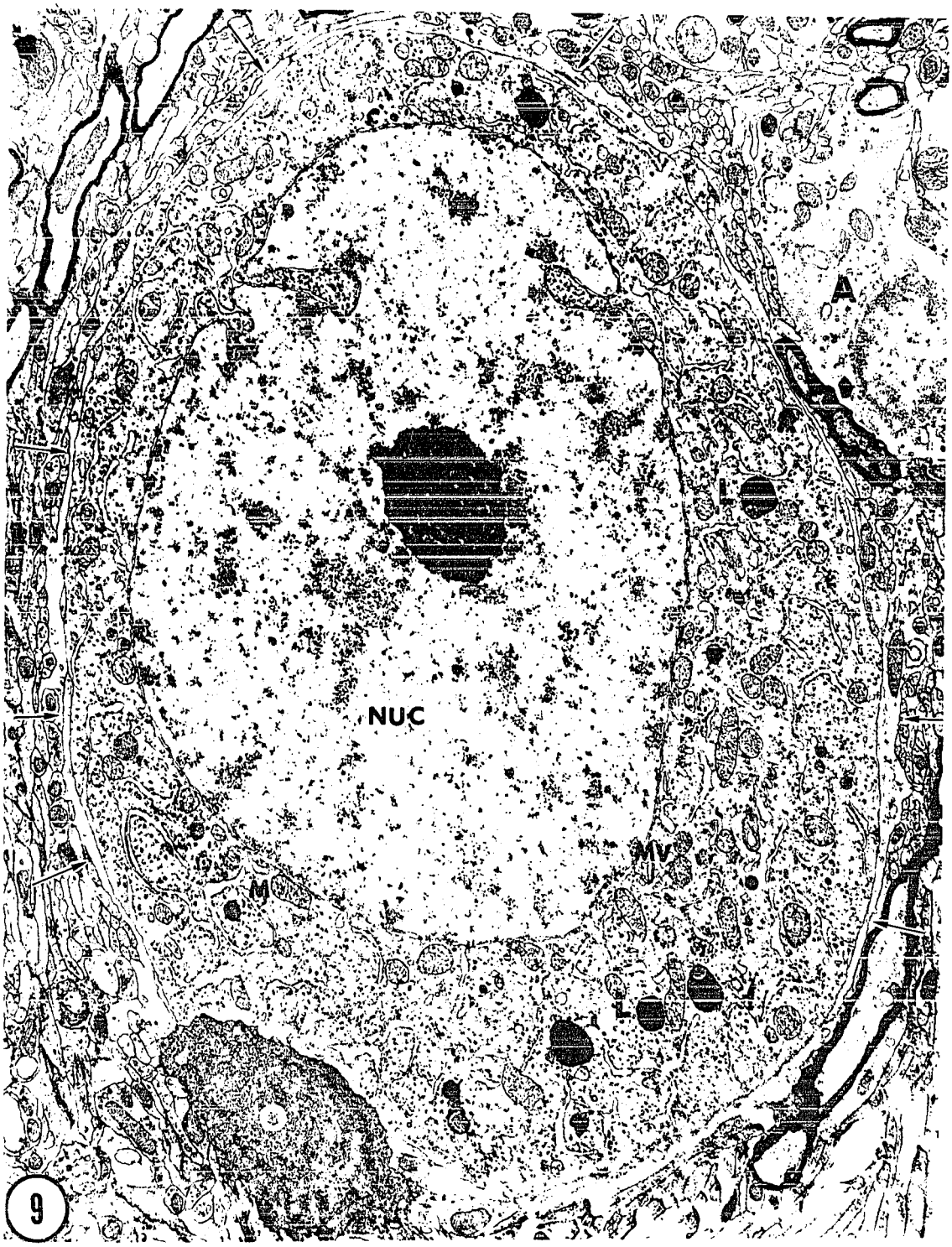
Protoplasmic astrocytes (fig. 9) and oligodendrocytes were identified in the two subdivisions of the central nucleus. The protoplasmic astrocytes were identified by the presence of bundles of cytoplasmic fibrils (according to the description by Peters et al. 1970). The karyoplasm of the nucleus stains lightly with condensations of chromatin material occuring around the nuclear envelope.

The oligodendrocytes were distinguished from astrocytes by the greater density of their cytoplasm and nucleus. The oligodendrocytes do not have fibrils or glycogen in their cytoplasm. This type of glial cell is commonly found in a satellite position. Because of its close association with the perikaryon of a nerve cell and the density of its nucleus, the dark cell in figure 9 was identified as an oligodendrocyte.

Fig. 9. Electron micrograph showing the cell body of a neuron in the medial division of the central amygdaloid nucleus. Note that the surface of the neuron is extensively covered by the processes of glial cells (at arrows).

Magnification: 7,000X

9


A
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9

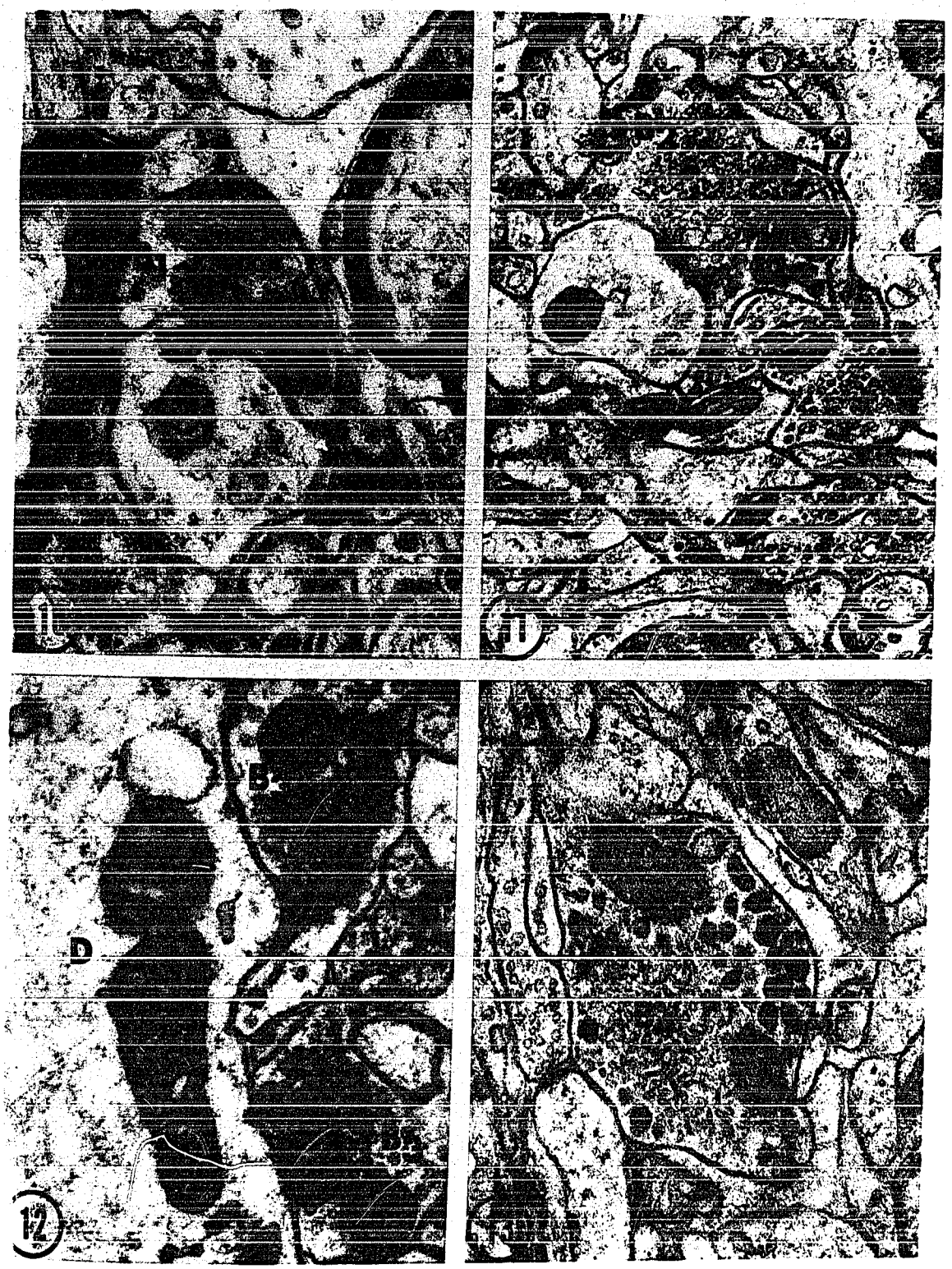
Electron micrographs illustrating the four major types of terminal boutons in the medial division of the central amygdaloid nucleus. Magnification of figures 10-13, 35,000X

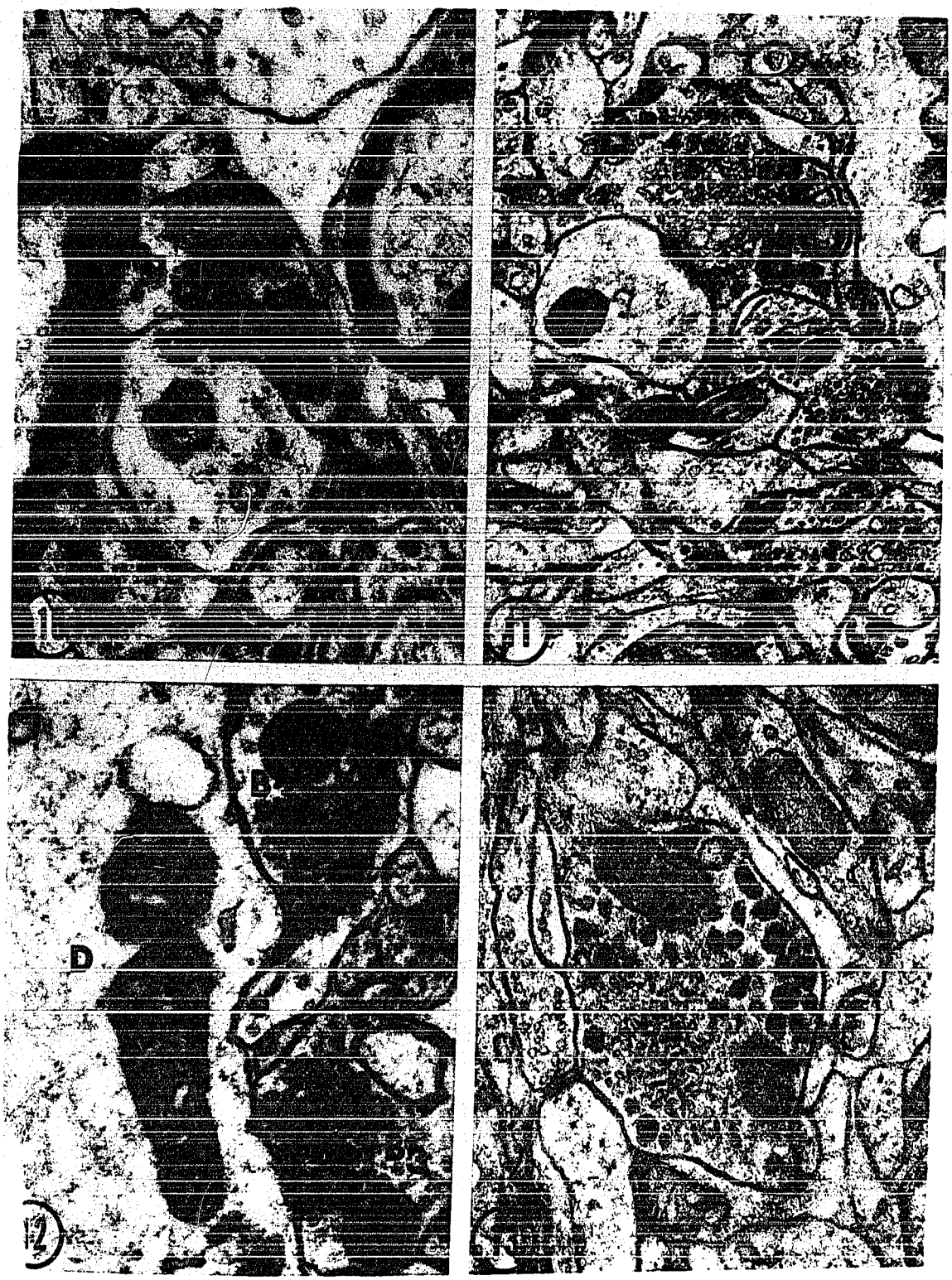
Fig. 10. Axodendritic synaptic contact formed by a Type 1 terminal bouton.

Fig. 11. Axodendritic contact formed by a Type 2 terminal bouton.

Fig. 12. Axodendritic contact formed by a Type 3 terminal bouton. (D, dendrite)

Fig. 13. Type 4 bouton containing many large dark-core vesicles.




1
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B
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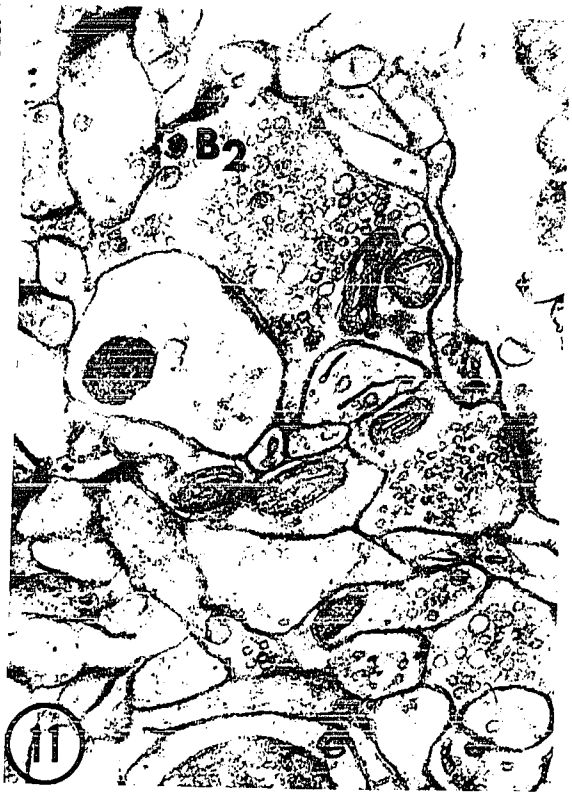
2

10



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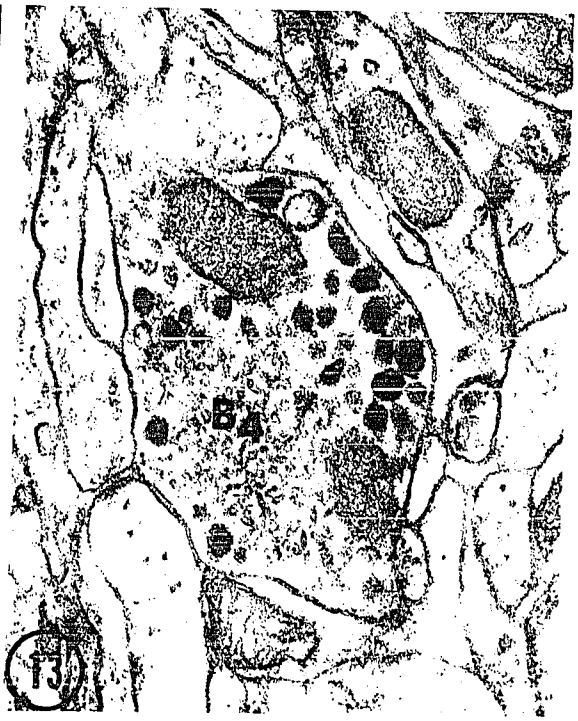


13

Fig. 14a. Electron micrograph of an axodendritic synaptic contact. Note the subsynaptic specialization indicated by the dark triangles.

Magnification: 35,000X

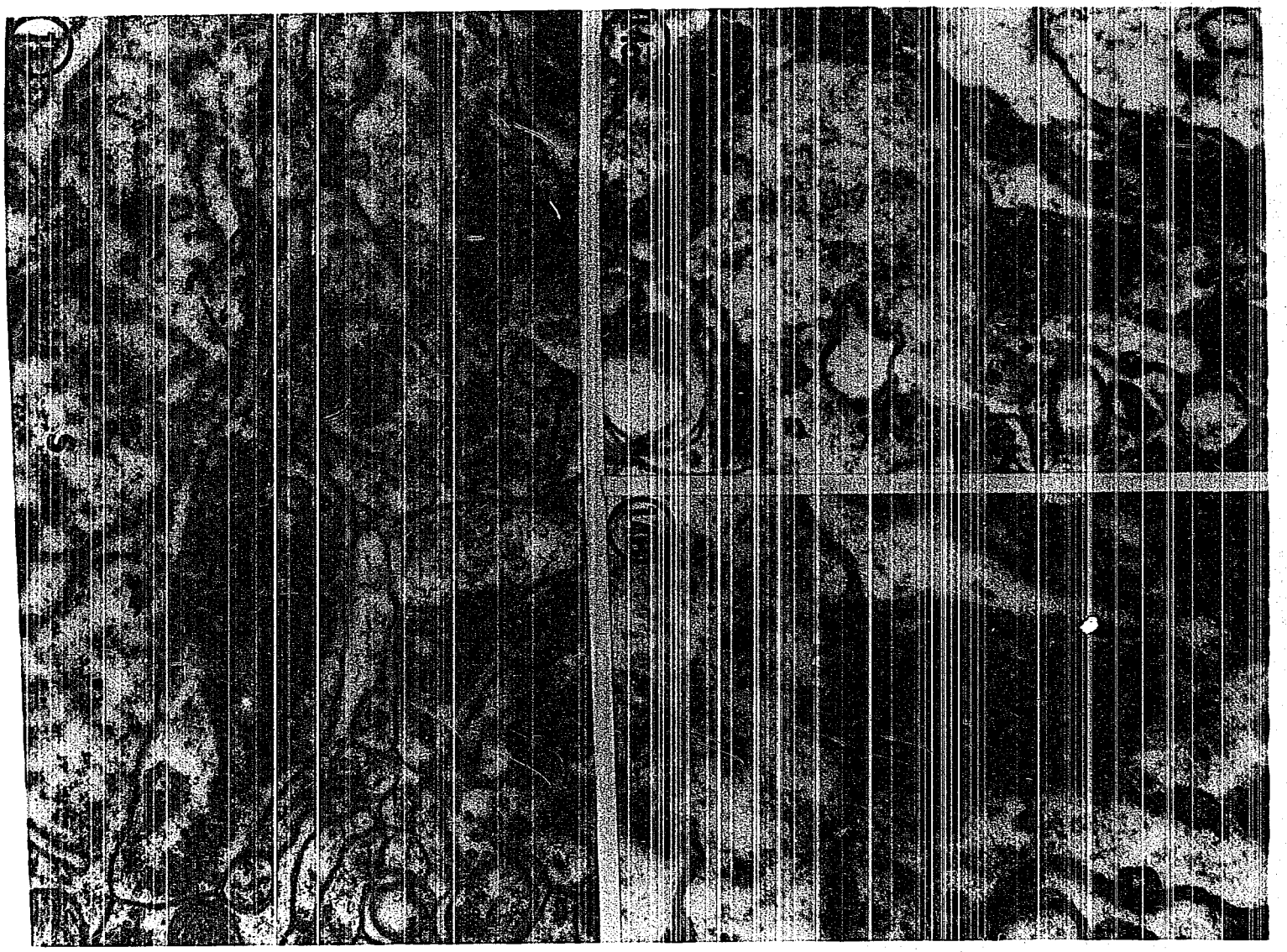
Fig. 14b. Higher magnification of the synaptic specialization shown in figure 14a.

Magnification: 70,000X

Fig. 15. Electron micrograph of an axosomatic synapse. Note the absence of a well defined postsynaptic density.

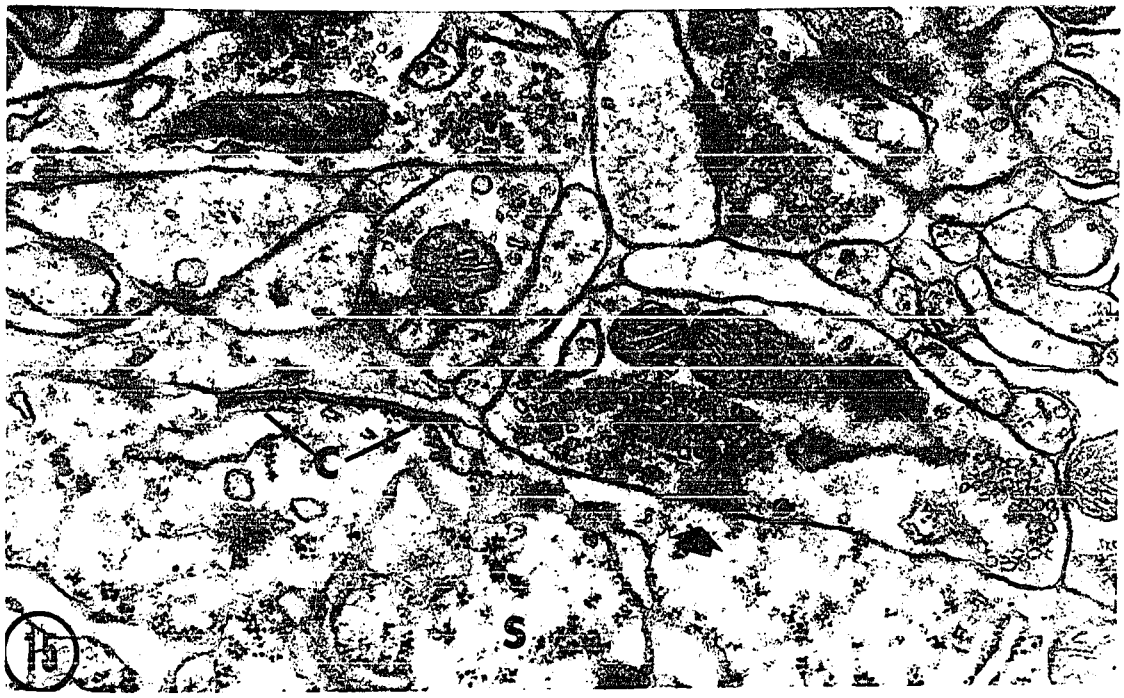
A subsurface cistern is shown to the left of the synaptic complex.

Magnification: 35,000X



14a

14b



15

Fig. 16. Electron micrograph of a cell body of a neuron in the lateral division of the central amygdaloid nucleus. Note the spine-like protrusion into the neighboring neuropil (⌂).

Magnification: 9,000X

NUC

G
M
NUC
16

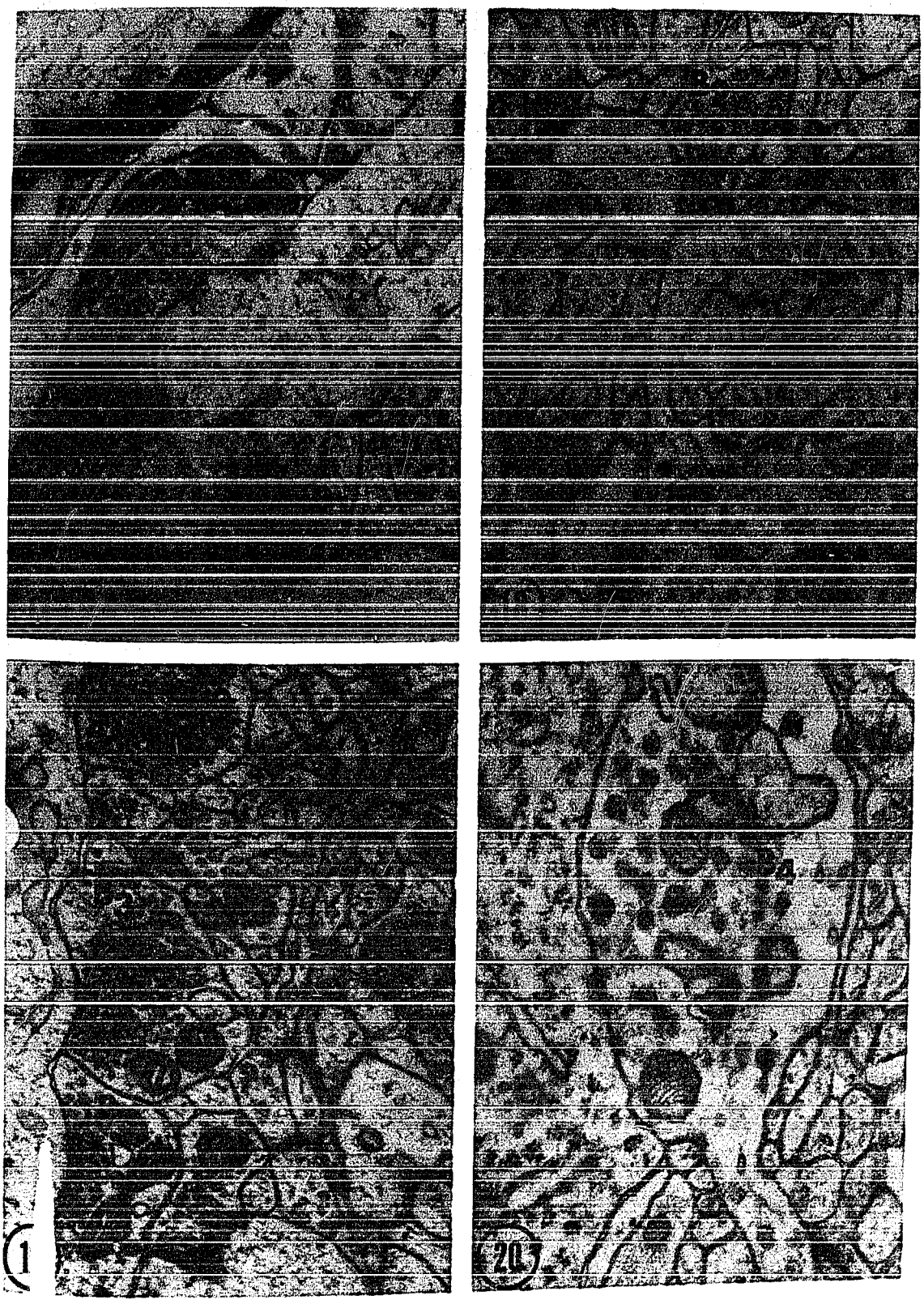
Electron micrographs illustrating the four major types of terminal boutons in the lateral division of the central amygdaloid nucleus. Magnification of figures 17-20, 35,000X

Fig. 17. Axodendritic contact formed by a Type 1 bouton.

Fig. 18. Axosomatic contact formed by a Type 2 bouton making synaptic contact with a soma spine.

Fig. 19. Axodendritic contact formed by Type 3 terminal boutons.

Fig. 20. Type 4 bouton containing predominately dark core vesicles.


(1
203

C) Electron microscopic observations of the putamen

No difference between the neurons in the central nucleus and the putamen could be determined on the basis of electron microscopic observations (compare figures 9 and 16 with figure 21).

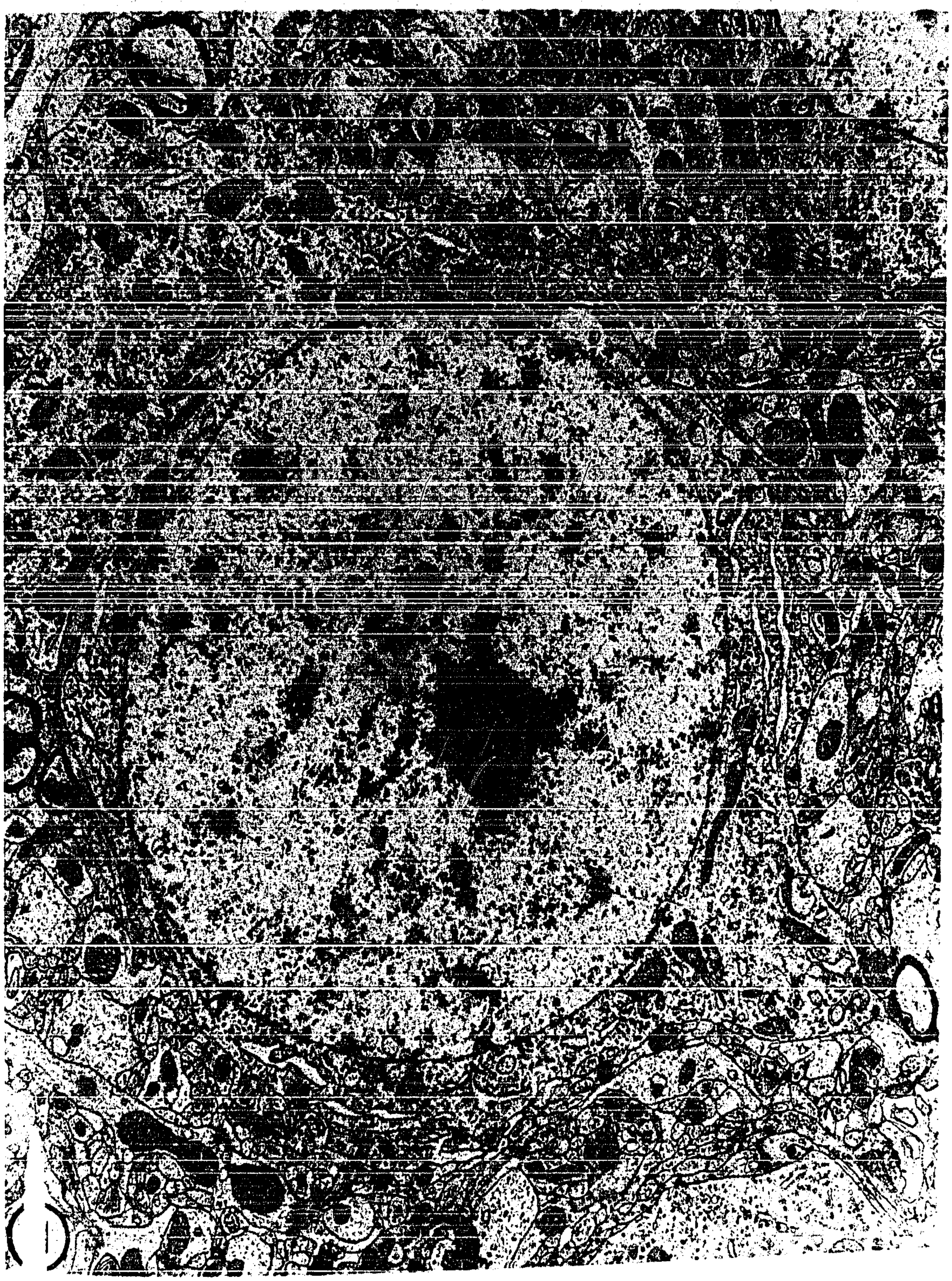
Terminal boutons with round synaptic vesicles are very frequently seen in the putamen. Examples of these boutons are shown in figures 22, 23 and 24. The vesicles in the presynaptic profile are round and of a uniform diameter. In the putamen, most of the synaptic contacts occur on dendritic spines and in many instances the spine could be traced back to its parent dendrite in the same micrograph (figs 23 and 24). Most of the spines contain a prominent spine apparatus. A small spine is seen in cross section in the lower left corner of figure 23. The postsynaptic profile seen in figure 22 was more difficult to identify. The cytoplasm contains a few filaments and possibly a few ribosomes. The profile is therefore considered to be a dendrite cut in cross section.

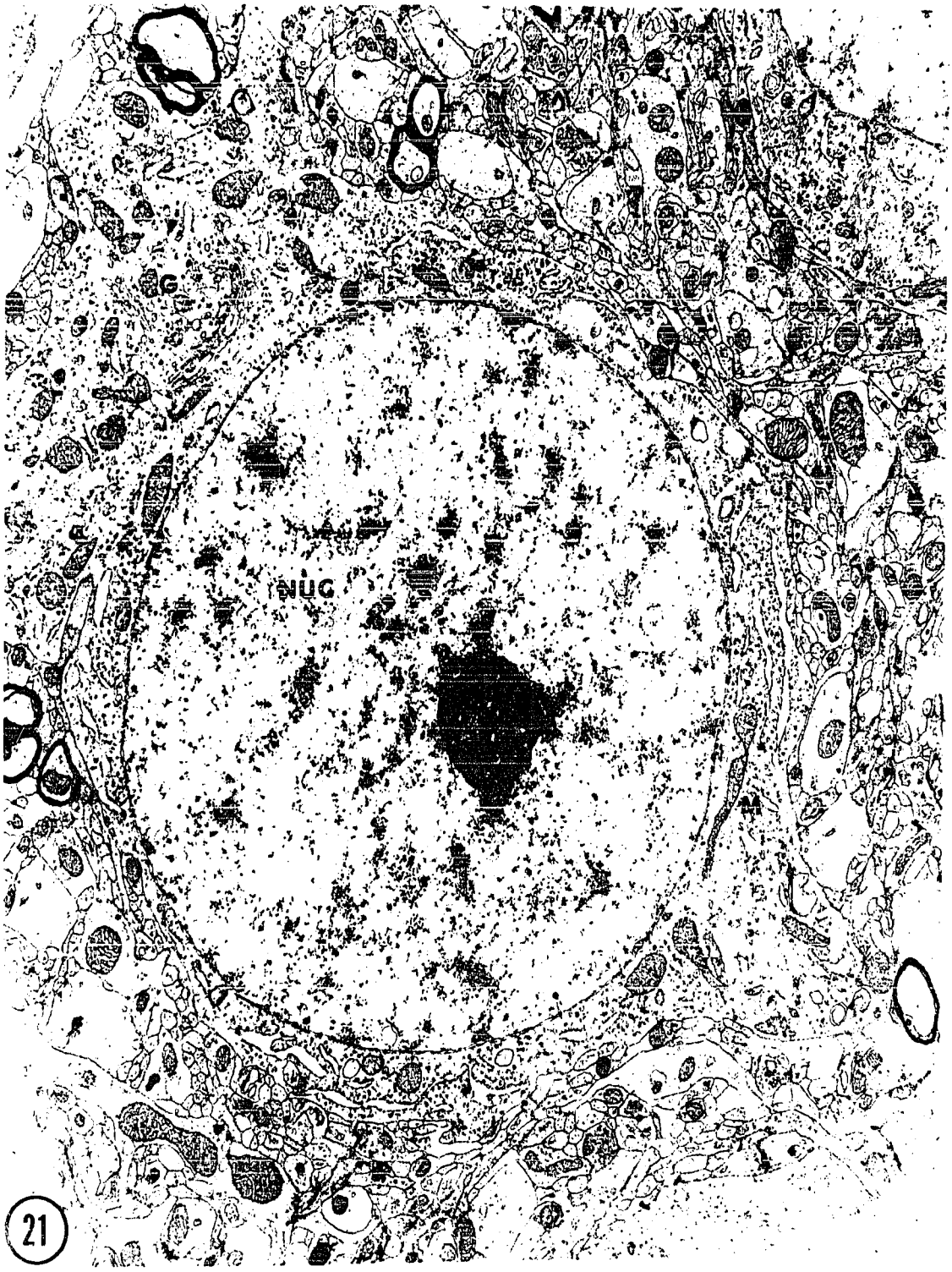
Boutons containing flattened vesicles are seldom seen in the putamen. This is in marked contrast to the situation in the central amygdaloid nucleus which has a very large population of boutons containing flattened vesicles.

No boutons containing predominately dark core vesicles were observed in the putamen.

Fig. 21. Electron micrograph showing a cell body of a neuron in the putamen.

Magnification: 6,000X





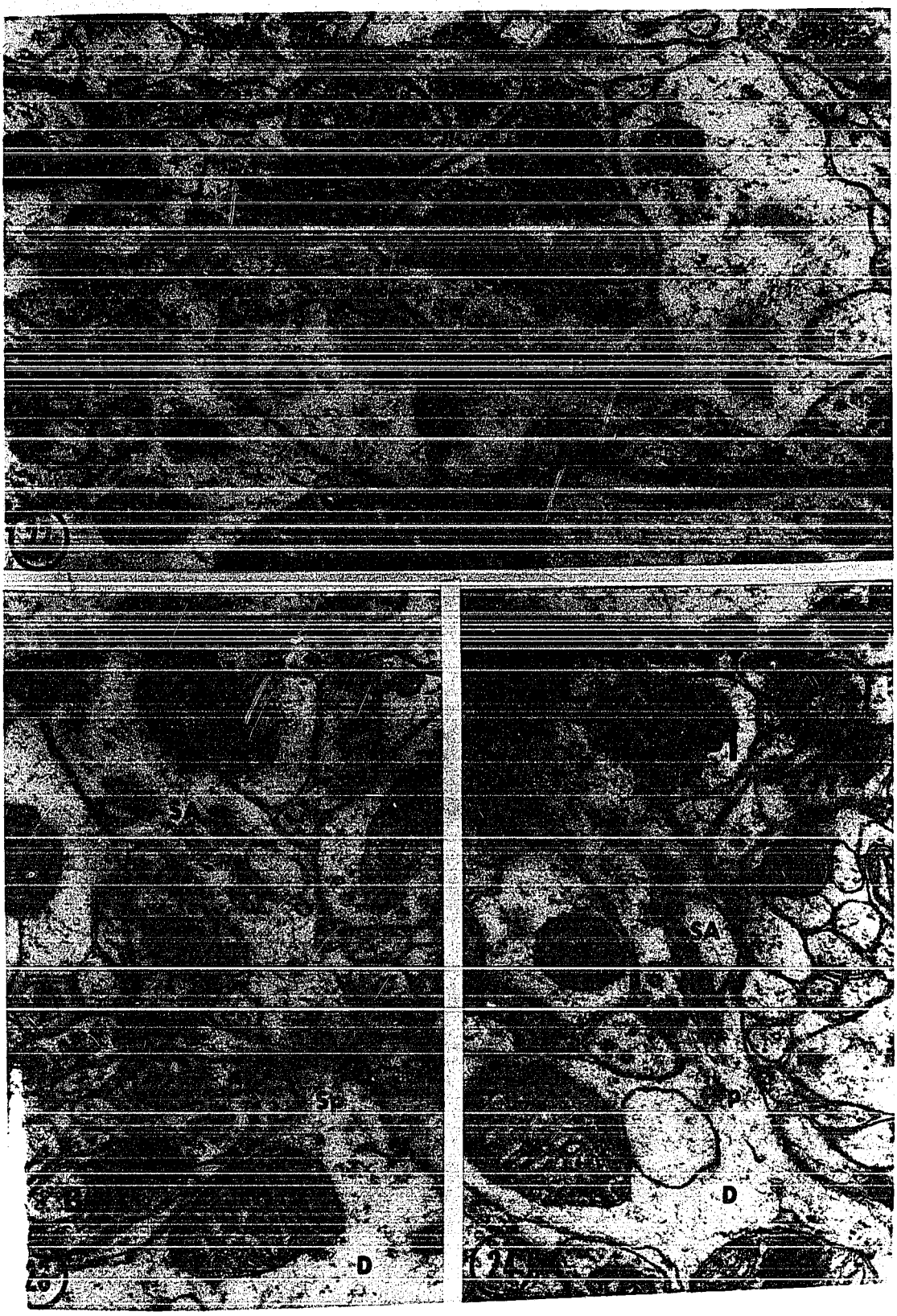
21

Electron micrographs illustrating the major type of terminal bouton in the putamen.

Magnification of figures 22-24, 35,000X

Fig. 22. Axodendritic contact formed by a Type 1 bouton.

Figs. 23 and 24. Axospinous contacts formed by a Type 1 bouton. Note that the spine may be traced back to the dendrite.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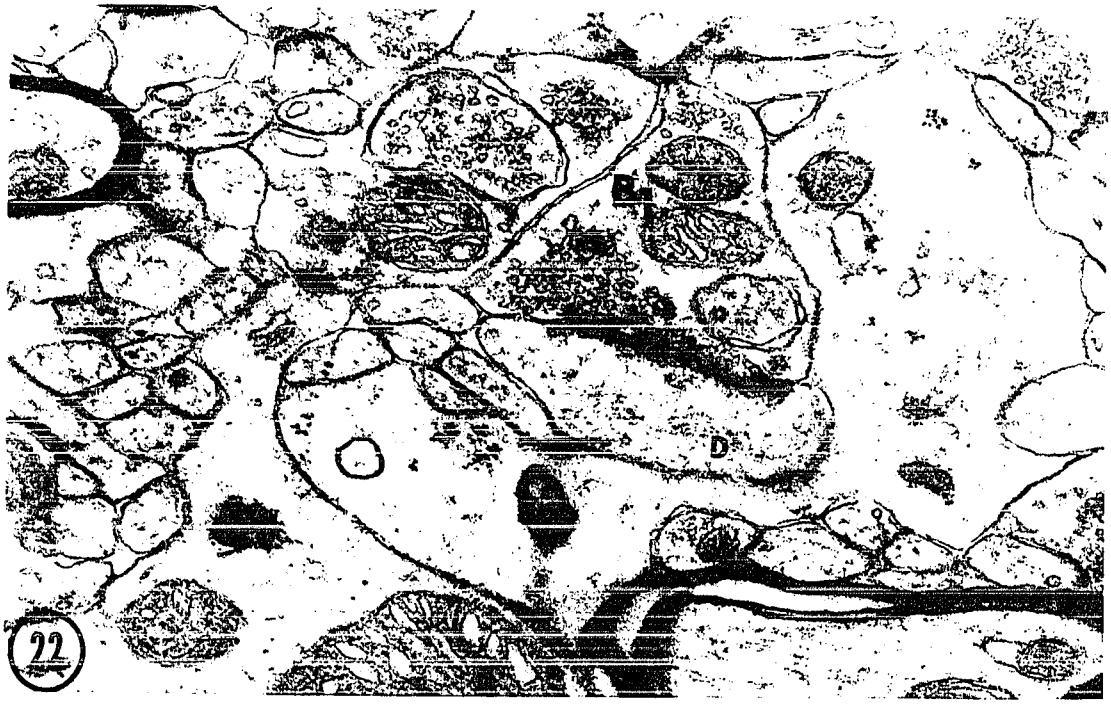

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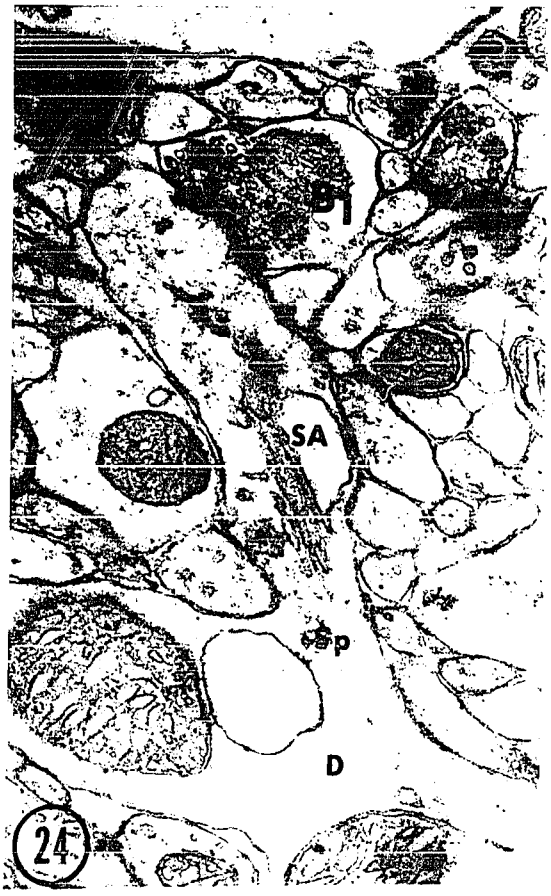


24

Experimental Material: Light microscopic observations

In the cat the hypothalamus lies in the floor of the diencephalon and is bounded rostrally by the septal and basal telencephalon and caudally by the mesencephalic tegmentum (Bleier, 1961). Le Gros Clark (1938) divided the hypothalamus of mammals into three regions, the pars supraoptica, the tuber cinereum and the pars mamillaris. He considered the preoptic area to be a part of the telencephalon but stated that it cannot be separated morphologically from the hypothalamus. This view has not been shared by subsequent investigators of the hypothalamus who have identified the preoptic area as a rostral differentiation of the hypothalamic anlage (Rose, 1942; Christ, 1969). The ontogenetic and phylogenetic studies supporting this conclusion have been reviewed recently by Christ (1969).

In the atlas of the cat hypothalamus, Bleier (1961) has adopted the nomenclature of Le Gros Clark but considered the preoptic area along with the supraoptic area to be one region, the anterior hypothalamic region. However, she retained the use of the term preoptic area for descriptive purposes. Bleier also divided the hypothalamus longitudinally into a medial and lateral division. In the cat, the medial division lies adjacent to the third ventricle and consists of several well defined

nuclei and a few more loosely grouped cellular portions which are termed areas. The lateral division is an extensive area of large cells which are scattered among the fibres of the medial forebrain bundle.

In the following experimental study, the terminology of Bleier has been used. Lesions were placed in those areas or nuclei of the hypothalamus known to receive fibres from the amygdaloid complex. These include the medial preoptic area and anterior and ventromedial nuclei of the medial division; and the lateral preoptic and hypothalamic areas of the lateral division.

I. Lesions of the Hypothalamus at the Tuberal Level

A) Lesions of the ventromedial nucleus (Cats 18 and 29)

There was very little degeneration resulting from these lesions. The lesion in cat 18 is limited to the rostral part of the ventromedial nucleus and is no more than 0.5 mm in diameter. No degeneration could be seen leaving this area. On the side contralateral to the lesion, abundant degeneration was observed in the vicinity of the electrode tract. Since fibre degeneration was stained along the electrode tract, the failure to demonstrate degeneration from the small lesion in the ventromedial nucleus cannot be due to the staining technique. More likely, the survival period of four days was too short.

When the lesion was large (fig. 25) and the survival period longer (8 days) a few fine degenerated fibres could be followed into the dorsal hypothalamic area (compare figs 26 and 27), the anterior hypothalamic, posterior hypothalamic and paraventricular nuclei. No degenerating fibres were traced into the medial forebrain bundle.

Fig. 25. Photographic enlargement of a section through the lesion in the ventromedial nucleus of the hypothalamus. (unstained section) Cat 29

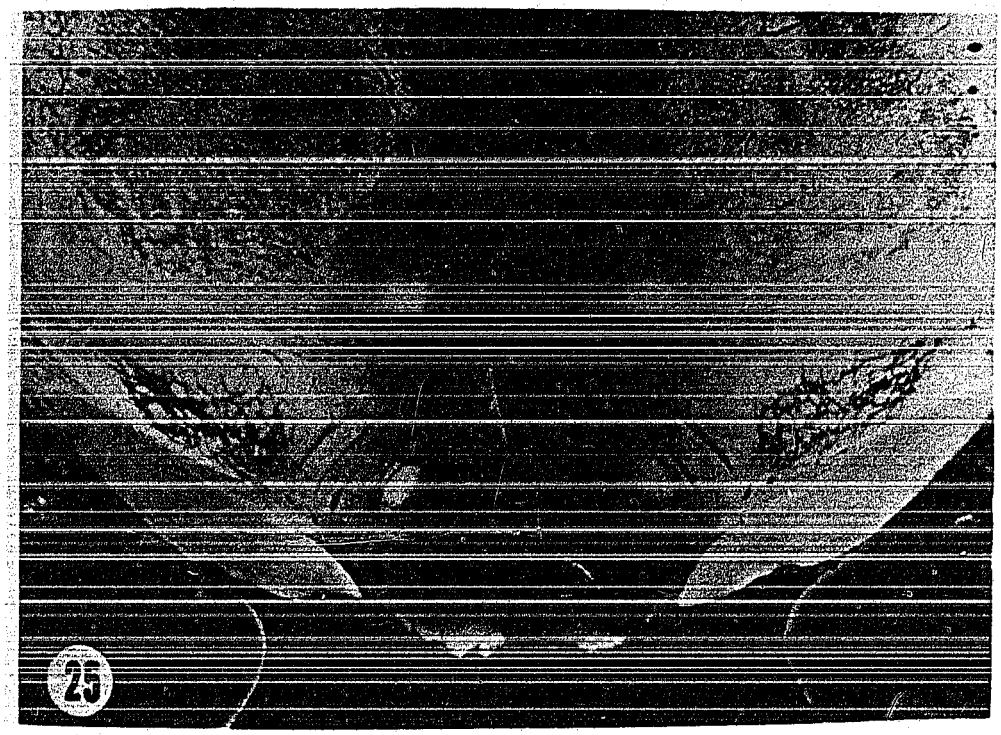
Magnification: 8X

Fig. 26. Light micrograph of the dorsal hypothalamic area on the side contralateral to the lesion shown in figure 25. Fink and Heimer stain, Procedure II.

Magnification: 500X

Fig. 27. Light micrograph of degenerated fibres and terminals in the dorsal hypothalamic area following a lesion to the ventromedial nucleus. Fink and Heimer stain, Procedure II.

Magnification: 500X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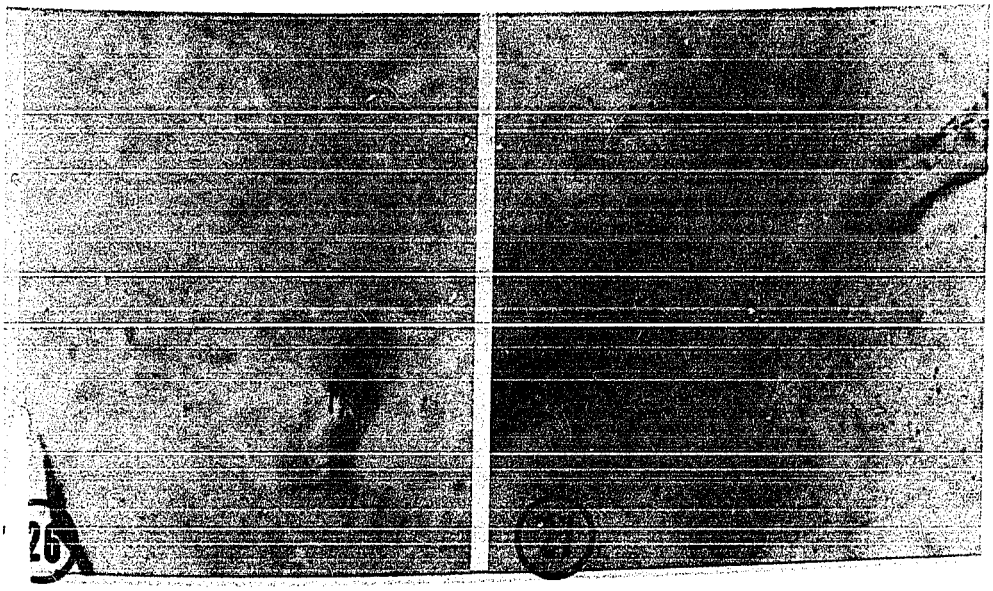
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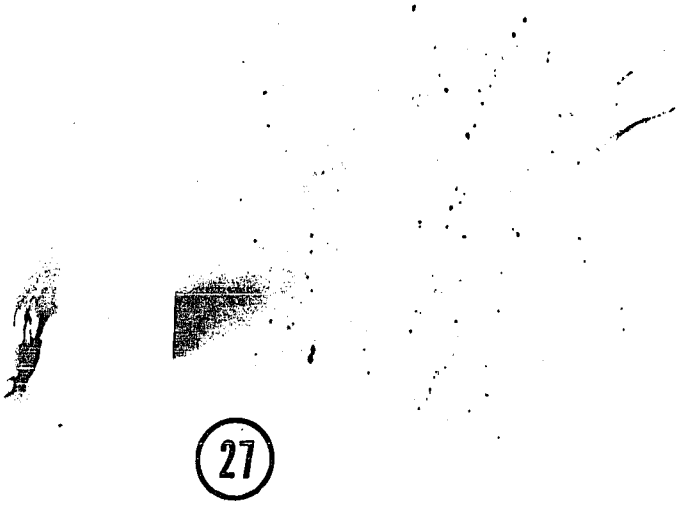

27

B) Lesions of the lateral hypothalamic area (Cats 26, 28 and 30)

All of these lesions interrupted the fibres of the medial forebrain bundle at the level of the tuber cinereum. One such lesion is shown in figure 28 from cat 26.

Degenerated fibres were traced caudally, rostrally, and dorsally from the lesion. The caudally directed fibres course as a component of the medial forebrain bundle to terminate in the anterior mammillary nucleus (compare figs 29 and 30) and in the medial and lateral mammillary nuclei of the hypothalamus. Terminal degeneration was also seen in the lateral hypothalamic area and surrounding the ventromedial nucleus (compare figs 31 and 32). Longer fibres course to the lateral preoptic area, medial and lateral septal nuclei, nucleus accumbens and globus pallidus. These fibres are shown diagrammatically in tracings of representative frontal sections from cat 28 in figure 33 A, B and C. The largest number of fibres turn rostrodorsally from the lesion to terminate in the medial thalamus (fig. 33 B, C and D). Terminal degeneration was seen in the parataenial and dorsomedial nuclei, nucleus reuniens and dorsal border of the nucleus ventralis anterior. Other fibres enter the stria medullaris to terminate in the

lateral nucleus of the habenula.

There were no degenerated fibres or terminals in the amygdaloid complex. Since degeneration to other areas of the brain stained well, the lack of any detectable degeneration in the amygdala was not due to technical failure.

Fig. 28. Photographic enlargement of a section through a lesion in the lateral hypothalamic area. (unstained) Cat 26

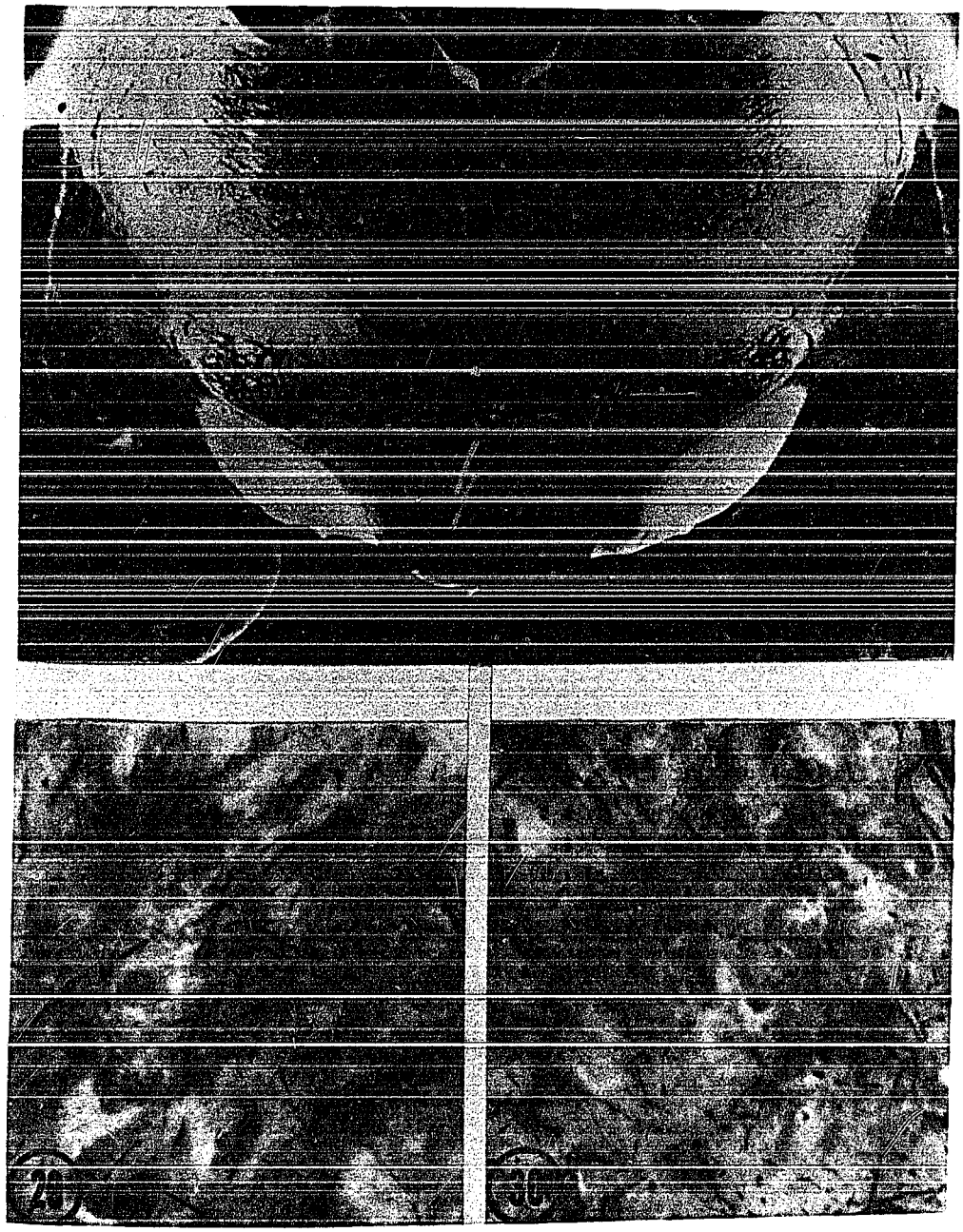
Magnification: 6½X

Fig. 29. Light micrograph of the anterior mammillary nucleus of the hypothalamus on the side contralateral to the lateral hypothalamic lesion. Cat 26, Nauta-Laidlaw stain.

Magnification: 600X

Fig. 30. Light micrograph showing degenerated fibres and a few fine terminals in the anterior mammillary nucleus of the hypothalamus following a lesion of the lateral hypothalamic area. Cat 26, Nauta-Laidlaw stain.

Magnification: 600X

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29


30

Fig. 31. Light micrograph illustrating the degenerated terminal endings surrounding the ventromedial border of the ventromedial hypothalamic nucleus following a lesion of the lateral hypothalamic area.

Cat 30, Wiitanen stain with a cresyl echt violet counterstain.

Magnification: 150X

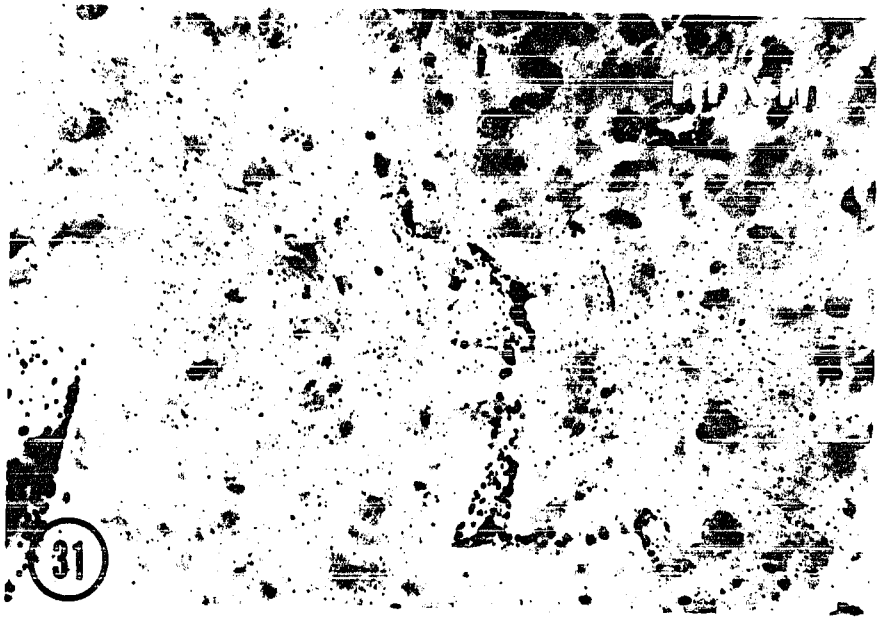
Fig. 32. Light micrograph of the ventromedial hypothalamic nucleus on the side contralateral to a lesion of the lateral hypothalamic area.

Cat 30, Wiitanen stain with a cresyl echt violet counterstain.

Magnification: 150X

nhvm

nhvm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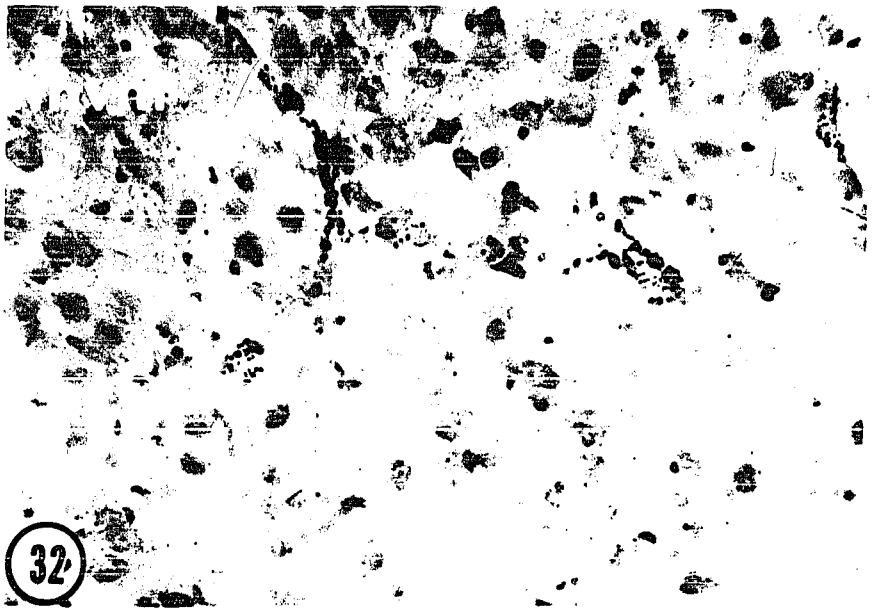
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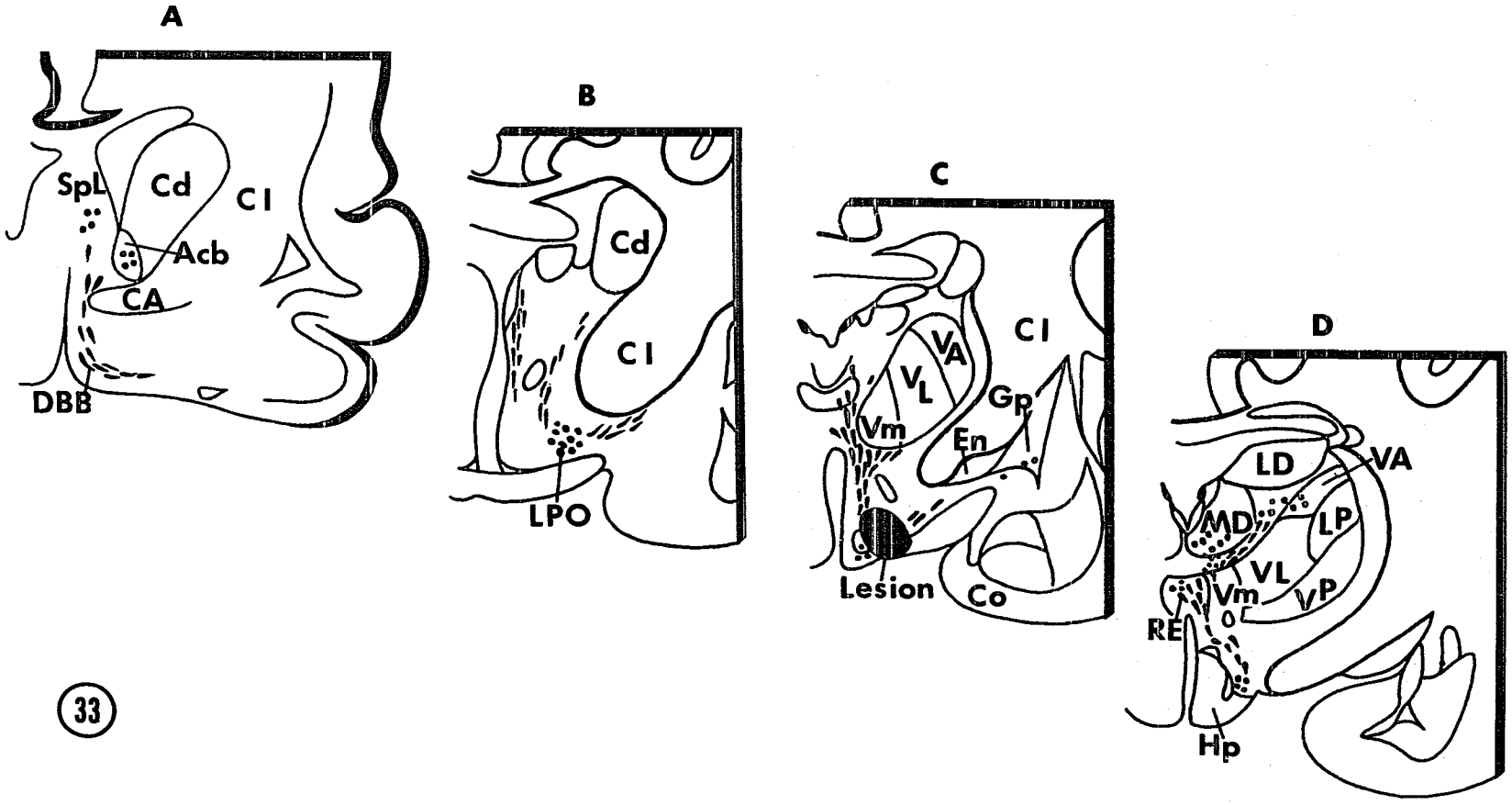
Fig. 33. Drawings of representative sections from cat 28 to illustrate the course and termination of degeneration following a lateral hypothalamic lesion.
The lines indicate degenerated fibres, the small round dots indicate terminal endings. Oblique frontal plane of section.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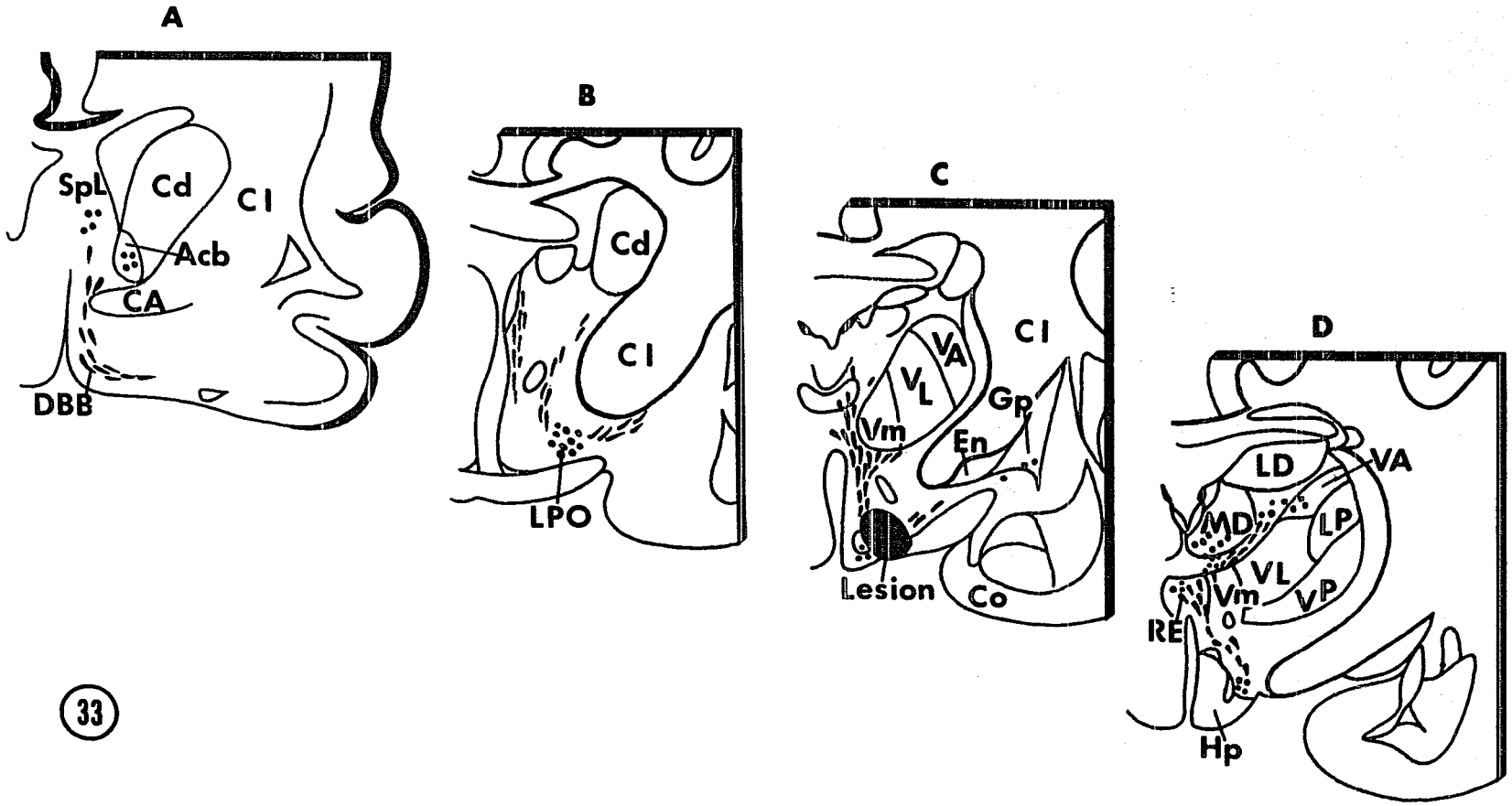
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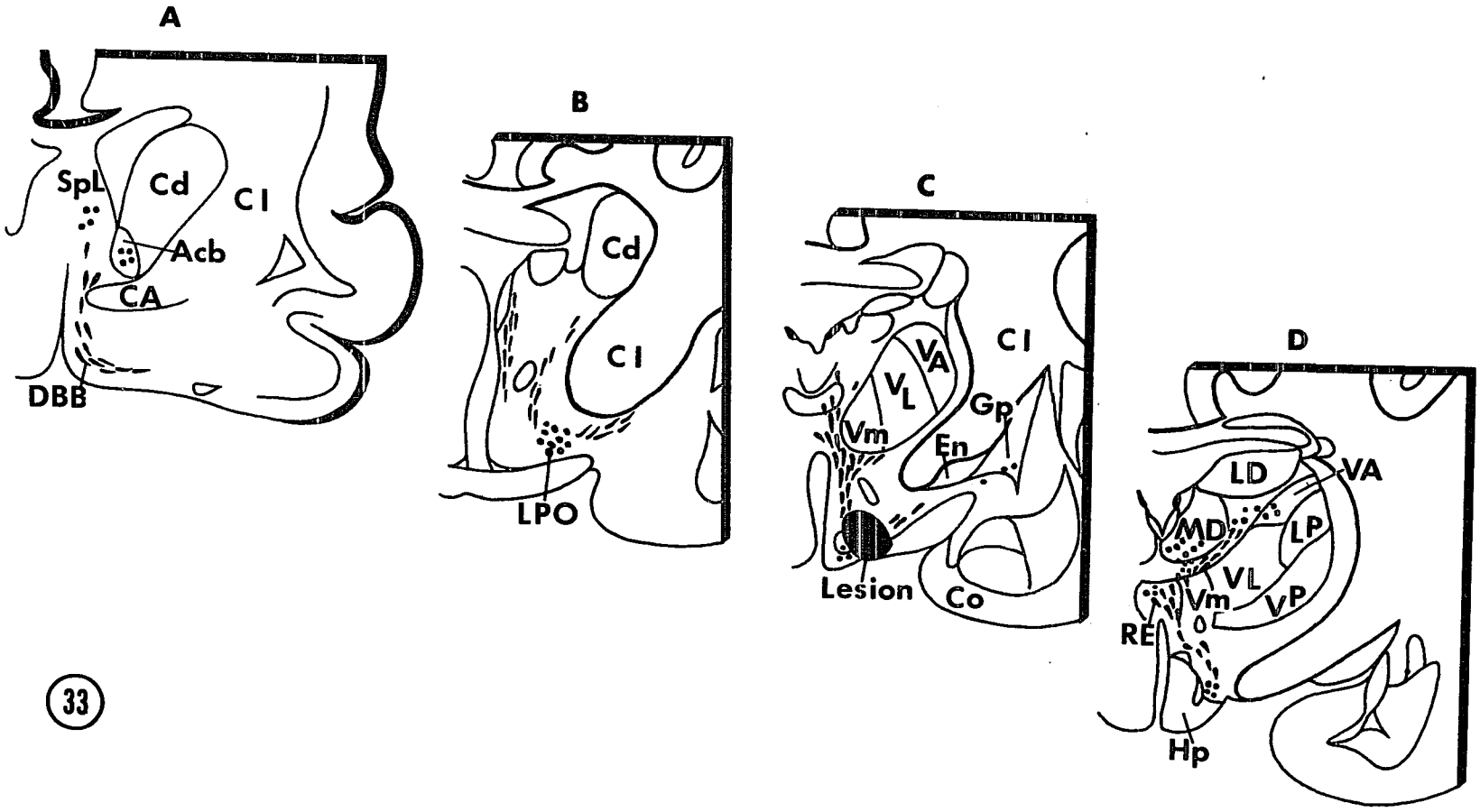
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II. Lesions of the Anterior Hypothalamus

A) Lesion of the anterior hypothalamic nucleus (Cat 25)

Because of the limited antero-posterior extent of the anterior hypothalamic nucleus, it was very difficult to localize this lesion. Only one cat had a well placed lesion which did not encroach upon either the preoptic area rostrally or the ventromedial nucleus caudally (fig. 34).

A few degenerated fibres could be followed from the lesion to the medial division of the central amygdaloid nucleus and to the basal and lateral amygdaloid nuclei. These fibres course through the lateral hypothalamus and turn lateralward at the level of the preoptic area. They continue lateralward, coursing above the optic tract to terminate in the amygdala. No degenerated fibres were seen in the stria terminalis.

Degenerated terminals were found in the medial preoptic area (compare figs 35 and 36) and in the ventromedial hypothalamic nucleus. The pattern of degeneration in the rest of the brain is the same as that shown in the diagram in figure 33 and will not be repeated here.

Fig. 34. Photographic enlargement of a section through the rostral portion of a lesion in the anterior hypothalamic nucleus. Cat 25.

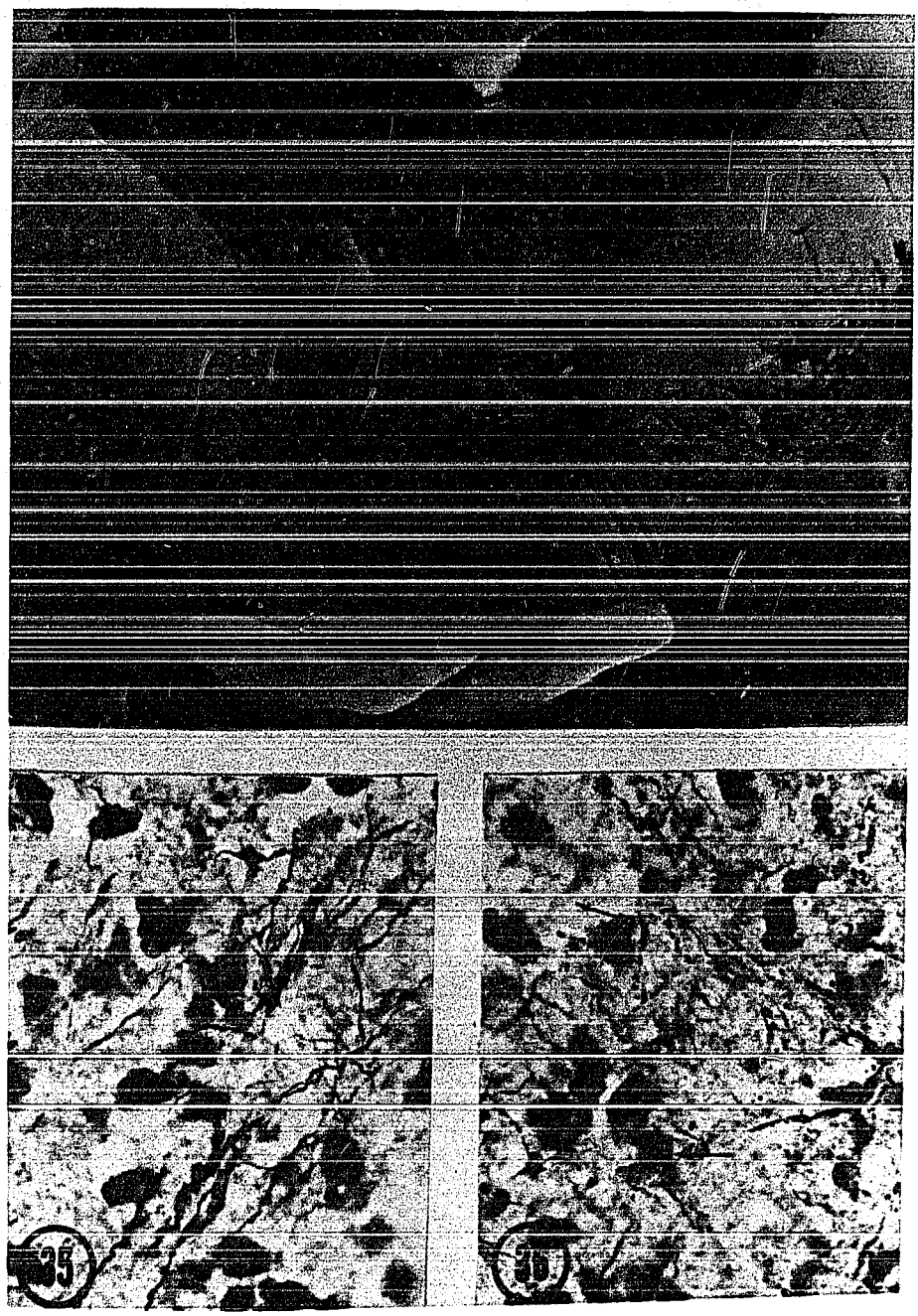
Magnification: 7X

Fig. 35. Light micrograph of the medial preoptic area on the side contralateral to a lesion of the anterior hypothalamic nucleus. Cat 25, Nauta-Laidlaw stain.

Magnification: 600X

Fig. 36. Light micrograph of degenerated terminals (at arrows) and fibres in the medial preoptic area of the hypothalamus following a lesion of the anterior hypothalamic nucleus. Cat 25, Nauta-Laidlaw stain.

Magnification: 600X

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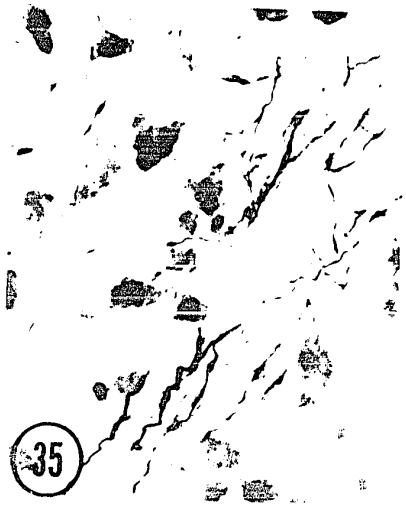

35


36

B) Lesions of the medial preoptic area (Cats CA 5, 32 and 19)

No degeneration to the amygdala was observed from any of the lesions of the medial preoptic area (figs 37 and 38). The distribution of degeneration to the mesencephalon was followed in the horizontal sections (cat 19). These fibres course from the lesion lateralward to enter the medial forebrain bundle. They continue caudally through the lateral hypothalamus to the midbrain tegmentum where they terminate (compare figs 39 and 40). Shorter degenerated fibres course from the lateral side of the lesion to terminate among the cells of the lateral preoptic area (compare figs 41 and 42).

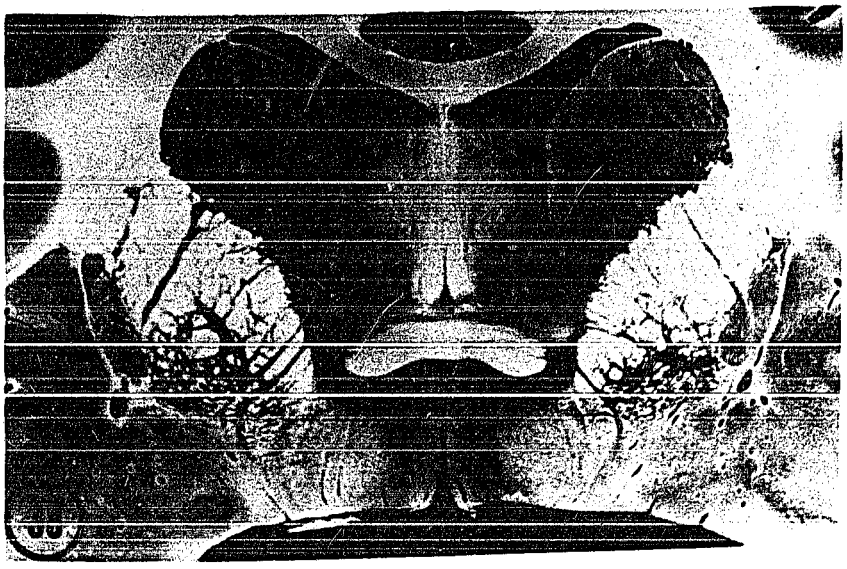
The distribution of the rest of the degeneration is the same as that shown diagrammatically in figure 33. No degenerated fibres enter the stria terminalis or the ventral pathway to the amygdala. The degeneration to other areas of the brain stained well so that degeneration to the amygdala was not missed due to the staining technique.

Fig. 37. Photographic enlargement of a section through the lesion in the superior portion of the medial preoptic area of the hypothalamus. (unstained) Cat CA 5

Magnification: 5½X

Fig. 38. Photographic enlargement of the lesion in the inferior portion of the medial preoptic area of the hypothalamus. (unstained) Cat 32

Magnification: 5½X



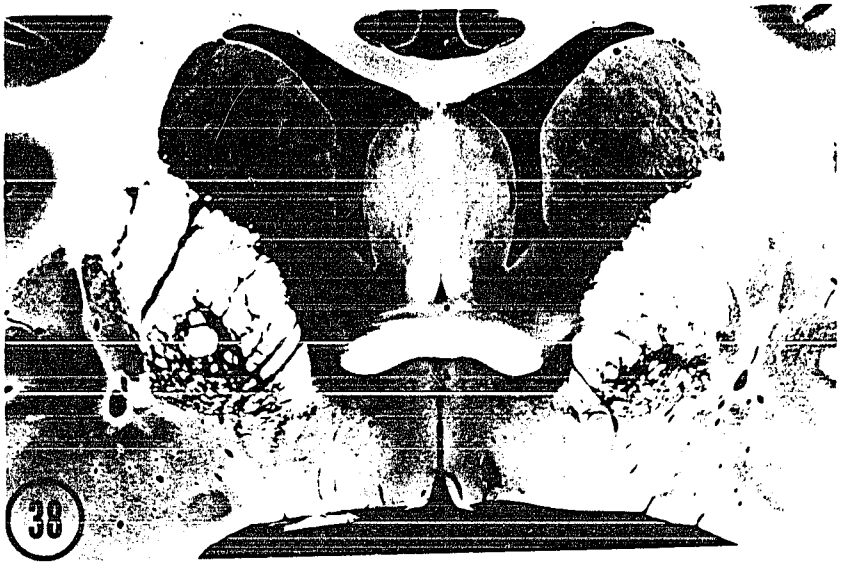

38

Fig. 39. Light micrograph of the mesencephalic tegmentum on the side contralateral to a lesion of the medial preoptic area. Cat 19, Wiitanen stain.

Magnification: 160X

Fig. 40. Light micrograph of the same area as in figure 40 but on the same side as the lesion.

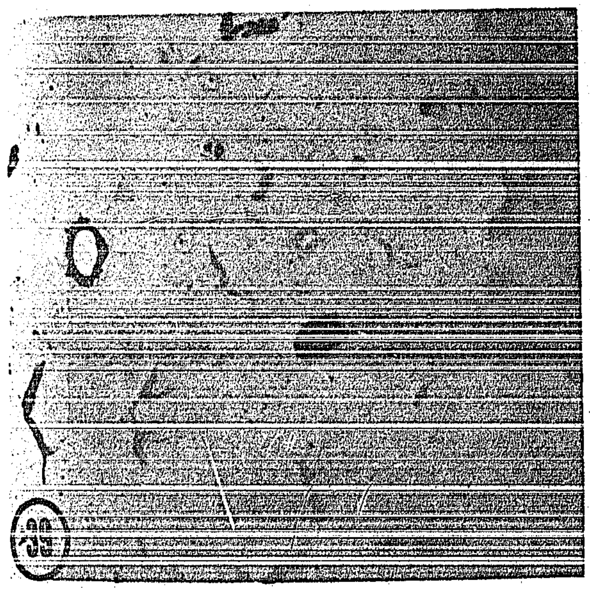
Magnification: 160X

Fig. 41. Light micrograph of the right lateral preoptic area following a lesion of the left medial preoptic area. Cat CA 5, Fink and Heimer stain, Procedure II.

Magnification: 400X

Fig. 42. Light micrograph of degenerated fibres and terminals in the left lateral preoptic area following a lesion of the left medial preoptic area. Cat CA 5, Fink and Heimer stain, Procedure II.

Magnification: 400X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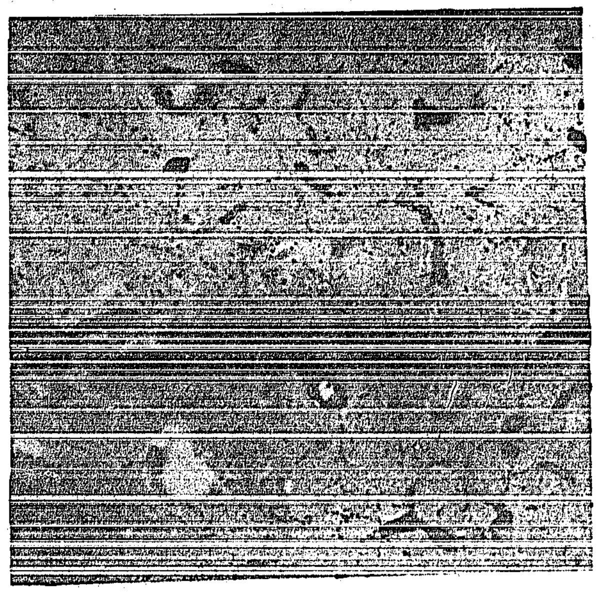

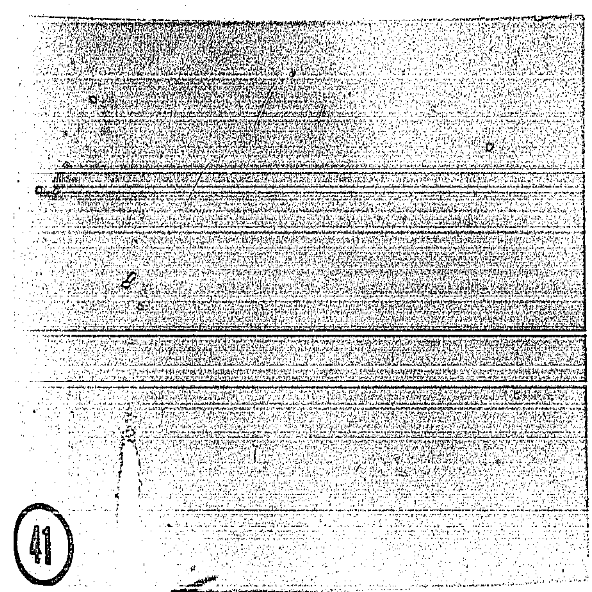
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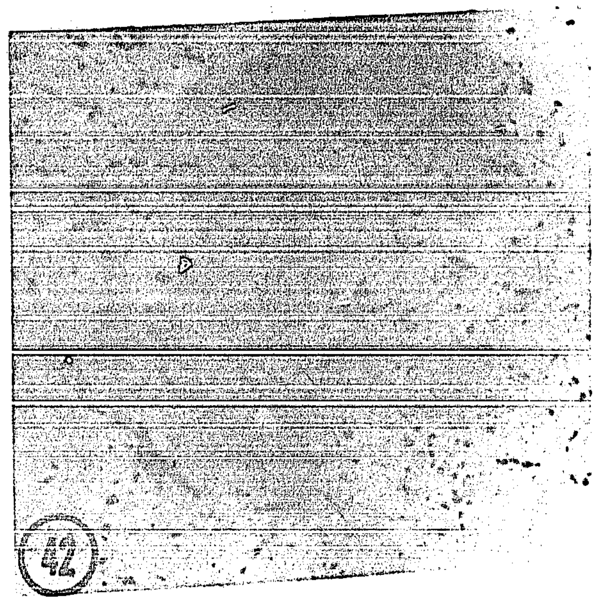

42

C) Lesions of the lateral preoptic area (Cats 1, 2, 3, 4, 5 and 6)

In producing these lesions, care was taken to avoid interrupting the fibres of the anterior commissure with the electrode tract. Therefore, the electrode was inserted from the contralateral side and angled slightly caudally. The electrode path is shown in figure 43 A to F. As seen in figure 43 A and B, the electrode passed through the frontal corona radiata, the head of the caudate nucleus (fig. 43 C and D) and the septum (fig. 43 E) on the side contralateral to the lesion. The electrode continued through the rostral portion of the medial preoptic area on the side ipsilateral to the lesion (fig. 43 F) before reaching its ultimate destination, the lateral preoptic area (figs 44, 45).

Degenerated fibres from the lesion in the lateral preoptic area enter both the stria terminalis and the ventral pathway. Some fibres that join the stria terminalis course anterior and others posterior to the anterior commissure to enter the ventral portion of the stria terminalis. The other group of fibres leaves the area of the lesion and courses laterally as a diffuse band of fibres above the optic tract (fig. 46 C and D). As this band of fibres turns ventralward at the level of the central amygdaloid nucleus it is joined by the fibres

Fig. 43 A to F. Photographic enlargements of representative frontal sections through the frontal pole of the brain to illustrate the electrode trajectory for the lateral preoptic lesions. (cat 3)

43
A
B
C
D
F

Fig. 44. Photographic enlargement of an unstained brain section showing a lesion of the lateral preoptic area. Cat 3, frontal plane of section.

Magnification: 5½X

Fig. 45. Photographic enlargement of an unstained brain section showing a lesion of the lateral preoptic area in a parasagittal section. Cat 4

Magnification: 5½X

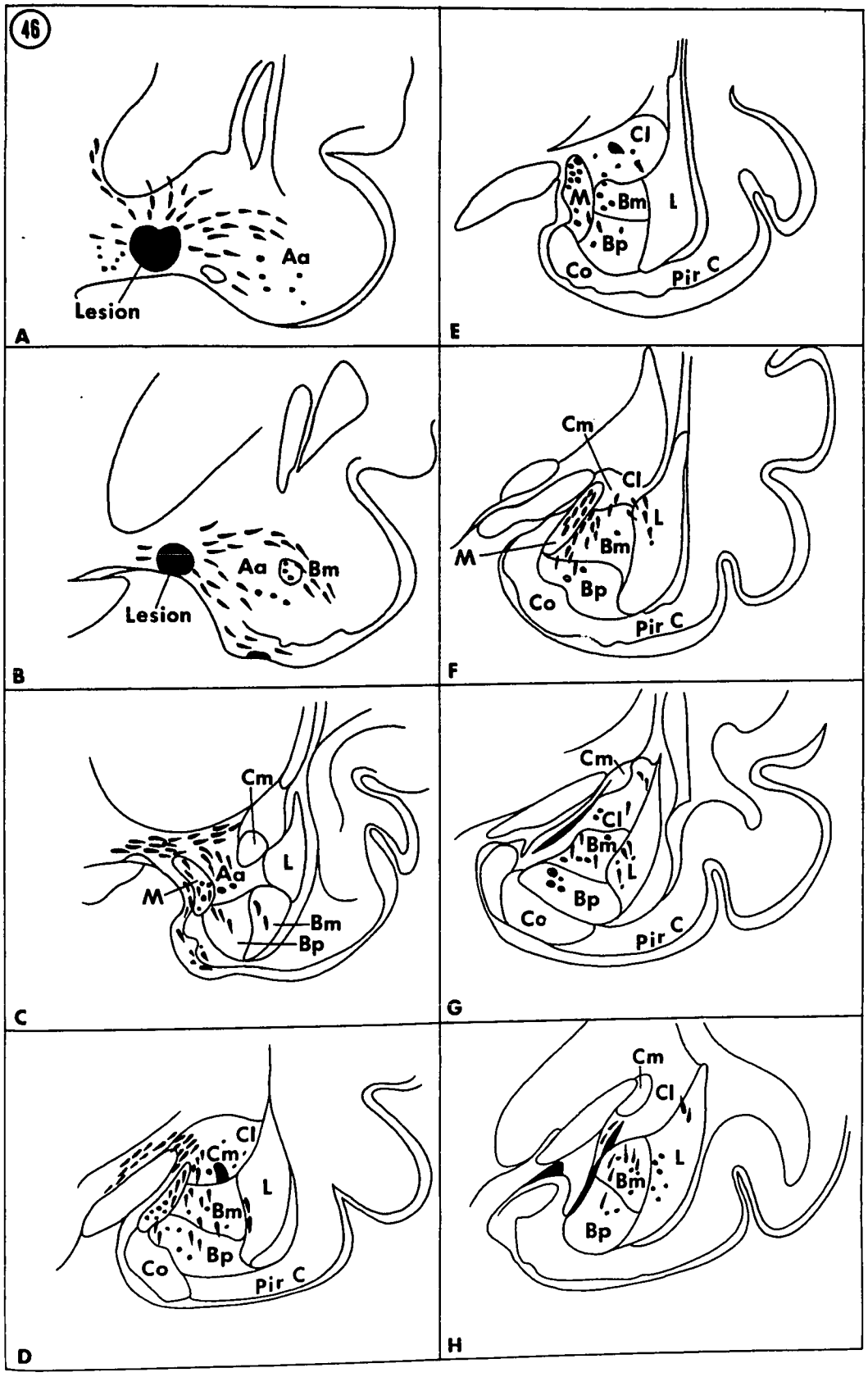

44
45

of the stria terminalis. These two pathways to the amygdala come together once they enter the central nucleus.

Scattered terminal degeneration is seen throughout the rostral two-thirds of the amygdaloid complex (fig. 46). In this region, a moderate amount of degeneration could be seen throughout the medial division of the central amygdaloid nucleus (compare figs 47 and 48) the medial amygdaloid nucleus (compare figs 51 and 52) and the anterior amygdaloid area (compare figs 59 and 60). The amount of degeneration in the lateral division of the central nucleus is very light and limited to its more medial portion (compare figs 49 and 50).

Only very light and scattered degeneration could be found in the basal and lateral amygdaloid nuclei (figs 53 to 58). Although the degeneration in the basal nucleus is not limited to any particular area, the degeneration in the lateral nucleus occurred mostly in the ventromedial portion at about the middle one-third of the amygdaloid complex (fig. 46 F to G). The presence of terminal degeneration in the lateral nucleus is difficult to ascertain. The fibres end abruptly and cannot be followed further in the same section or in adjacent sections.

Fig. 46. Drawings of representative sections through the rostral two-thirds of the amygdaloid complex to show degenerated fibres and terminals in the individual nuclei. Cat 3 Lateral preoptic area lesion. (the lines indicate degenerated fibres, the dots indicate degenerated terminal endings)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Aa
Lesion
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Aa
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Light micrographs of sections from the corticomedial group of amygdaloid nuclei following a lesion of the lateral preoptic area.

Magnification of all micrographs is 560X

Fig. 47. Micrograph of the medial division of the central nucleus on the side contralateral to the lesion. Cat 2, Nauta-Laidlaw stain.

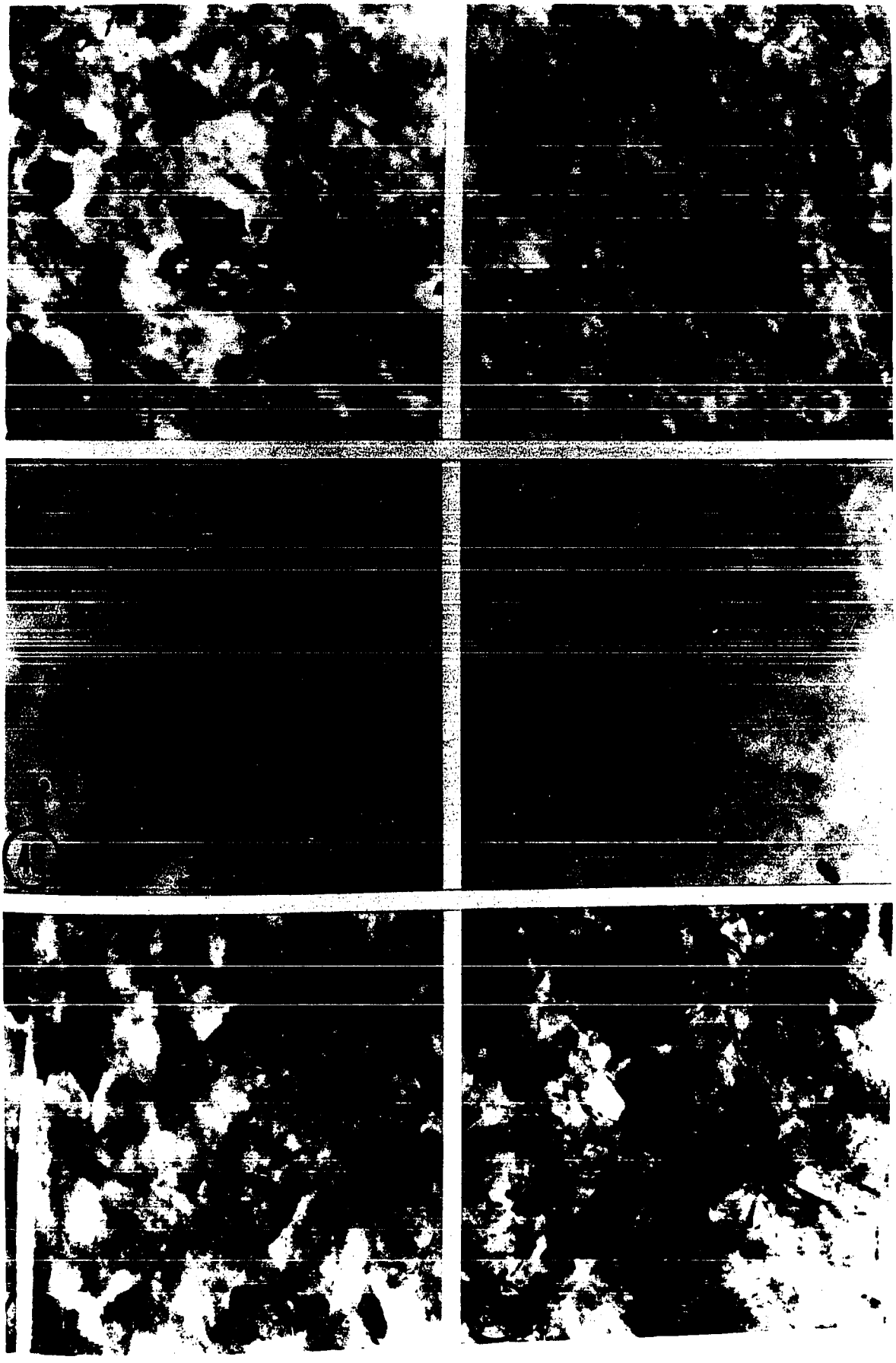
Fig. 48. Micrograph of the medial division of the central nucleus on the same side of the lesion showing the presence of degenerated fibres and terminals. Cat 2, Nauta-Laidlaw stain.

Fig. 49. Micrograph of the lateral division of the central nucleus on the side contralateral to the lesion. Cat 1, Fink and Heimer stain, Procedure II.

Fig. 50. Micrograph of the lateral division of the central nucleus on the same side of the lesion showing the presence of degenerated fibres and terminals. Cat 1, Fink and Heimer stain, Procedure II.

Fig. 51. Micrograph of the medial nucleus on the side contralateral to the lesion. Cat 2, Nauta-Laidlaw stain.

Fig. 52. Micrograph of the medial nucleus on the same side of the lesion showing the presence of degenerated fibres and terminals. Cat 2, Nauta-Laidlaw stain.



Light micrographs of sections from the basolateral group of amygdaloid nuclei following a lesion of the lateral preoptic area.

Magnification of all micrographs is 560X

Fig. 53. Micrograph of the large-celled portion of the basal nucleus on the side contralateral to the lesion. Cat 4, Nauta-Laidlaw stain.

Fig. 54. Micrograph of the large-celled portion of the basal nucleus on the same side as the lesion showing the presence of degenerated fibres and terminals. Cat 4, Nauta-Laidlaw stain.

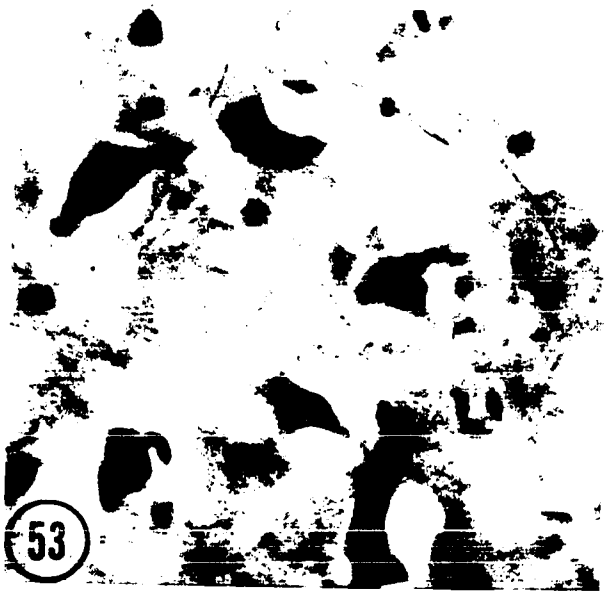
Fig. 55. Micrograph of the small-celled portion of the basal nucleus on the side contralateral to the lesion. Cat 3, Nauta-Laidlaw stain.

Fig. 56. Micrograph of the small-celled portion of the basal nucleus on the same side as the lesion showing the presence of degenerated fibres and terminals. Cat 3, Nauta-Laidlaw stain.

Fig. 57. Micrograph of the lateral nucleus on the side contralateral to the lesion. Cat 4, Nauta-Laidlaw stain.

Fig. 58. Micrograph of the lateral nucleus on the same side as the lesion showing the presence of degenerated fibres. Cat 4, Nauta-Laidlaw stain.

5c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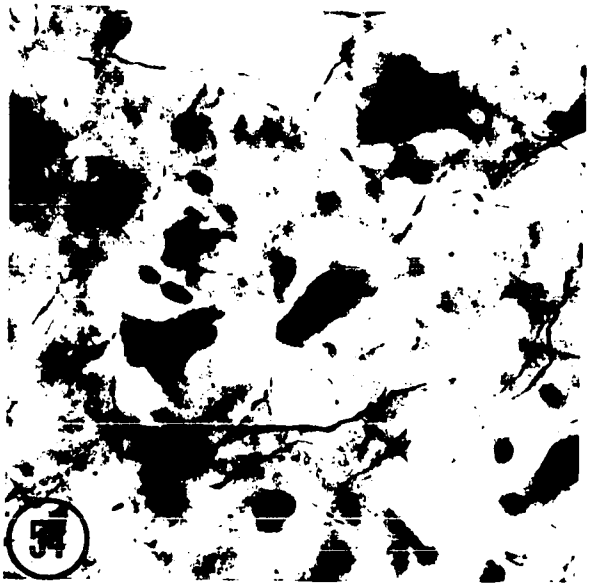
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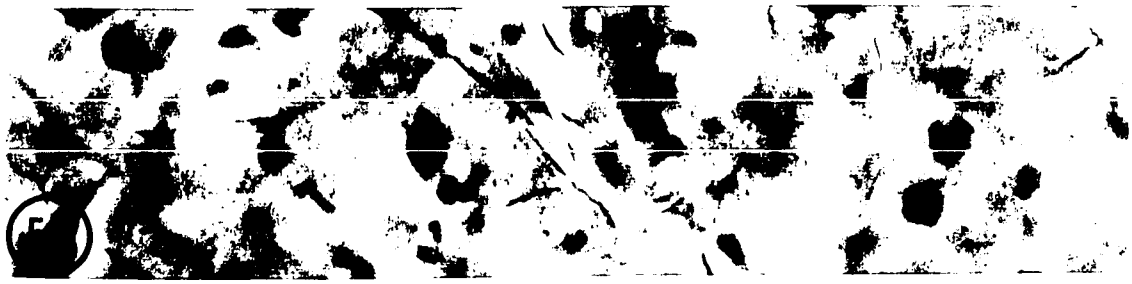


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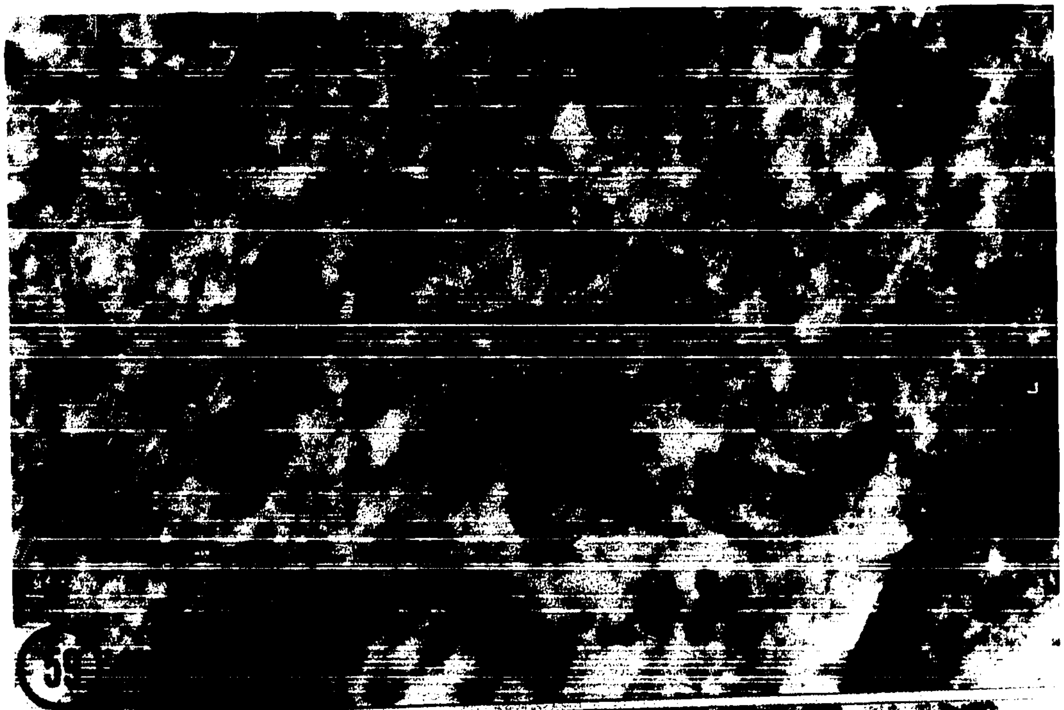
Fig. 59. Light micrograph of the anterior amygdaloid area on the side contralateral to a lesion of the lateral preoptic area. Cat 1, Fink and Heimer stain, Procedure I.

Magnification: 800X

Fig. 60. Light micrograph of the anterior amygdaloid area on the same side as a lesion of the lateral preoptic area showing the presence of degenerated fibres and terminals. Cat 1, Fink and Heimer stain, Procedure I.

Magnification: 800X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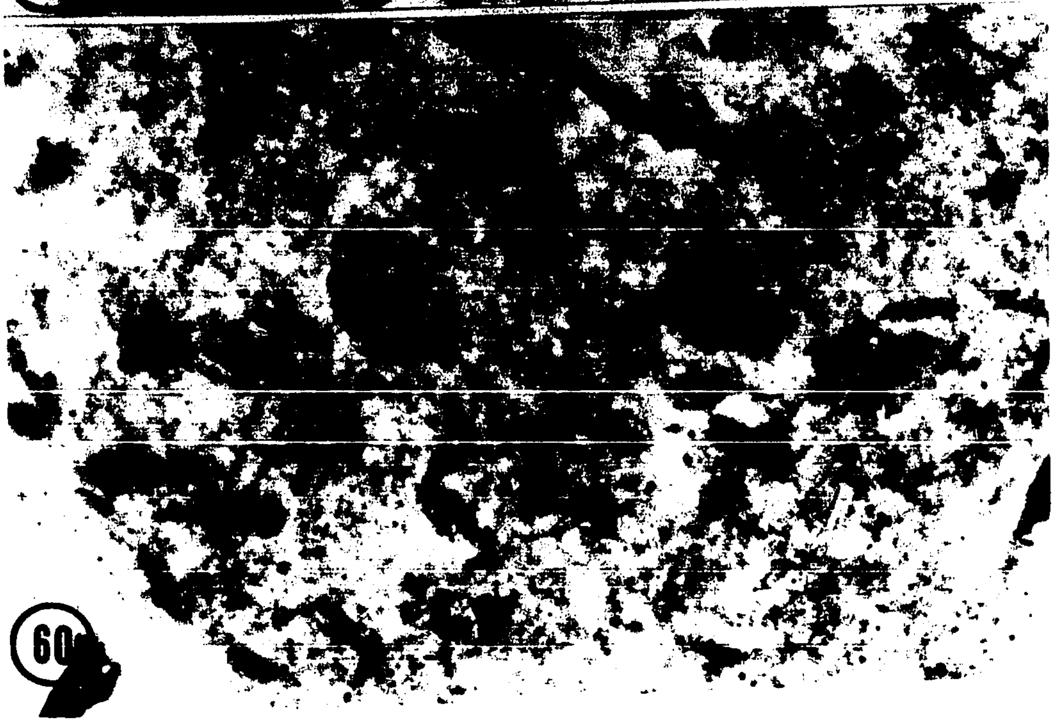
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The degeneration to the rest of the brain is similar in distribution to that seen after a more caudal lesion of the lateral hypothalamus and is summarized in the diagrams in fig. 33.

When the lesion is limited to the superior portion of the lateral preoptic area, the amount of degeneration in the amygdala is much less than that from the inferior portion. A few fibres course to the central, medial and basal nuclei by way of the stria terminalis. No detectable degeneration occurred in the ventral pathway to the amygdala.

Experimental Material: Electron microscopic observations (Cats EEM 1, 2, 3, 4 and 5)

The presence of degenerated terminal endings in the amygdaloid nuclei following a lesion of the lateral preoptic area was verified with the electron microscope. A small number of electron dense boutons was observed in the central amygdaloid nucleus (figs 61, 62 and 64), the medial amygdaloid nucleus (fig. 63), the basal amygdaloid nuclei (figs 66 and 68) and the lateral amygdaloid nucleus (fig. 67). No degeneration was located in the cortical amygdaloid nucleus in confirmation of the results of the light microscopic studies.

The appearance of the degenerated terminals varied according to the length of the post-operative survival time. The following account is a general description of the changes occurring within the boutons with longer survival times. It must be noted, however, that within each survival period boutons could be found in a state of degeneration more or less advanced than the 'typical' stages shown in the electron micrographs.

2-day stage (Cat EEM 1). A few terminal boutons show signs of early degeneration as indicated by an increase in the electron density of the matrix of the bouton.

3-day stage (Cat EEM 2). The degenerated boutons are similar to those of the two day stage but the synaptic vesicles are more closely packed. The vesicles are in most cases the flattened variety which are seen in the type 3 boutons of the normal central nucleus. Occasionally a type 1 or a type 2 degenerated bouton is seen (fig. 66) and in these the matrix within the terminal knob is much more electron dense than in the type 3 bouton. The mitochondria show no obvious signs of fragmentation or disruption.

5-day stage (Cat EEM 3). The matrix of the degenerated bouton at this stage is noticeably darker and more granular in appearance than in the normal bouton (figs 61 and 67). The axoplasm within the myelinated portion of the axon is also very electron dense (fig. 65). The outer membranes of the mitochondria are very indistinct and the cristae cannot be distinguished easily (fig. 62). In most cases the vesicles, when distinguishable, are flattened.

7-day stage (Cat EEM 4). Some boutons similar to the 5-day stage are found (fig. 63). Others are very

electron dense and the type of vesicle cannot be identified.

15-day stage (Cat EEM 5). The membrane of the bouton now shows many irregularities or wrinkles suggestive of a shrinkage of the synaptic knob (fig. 68). Synaptic clefts are still visible. Occasionally an astrocytic glial process containing filaments and glycogen particles is seen to encroach upon the bouton. Since this is also observed in normal material it cannot be determined if this is a phagocytic reaction.

As indicated in the electron micrographs, the shape of the vesicles in the electron dense profiles was nearly always of the flattened variety. This was true even in the medial and lateral nuclei in which the predominant vesicle population was of the round variety.

Degenerated axons of small to medium size were observed in all of the amygdaloid nuclei with the exception of the cortical nucleus. The axoplasm and mitochondria of the degenerated axons became more electron dense as the survival time was increased. No changes in the structure of the myelin sheath were noted at any stage of degeneration..

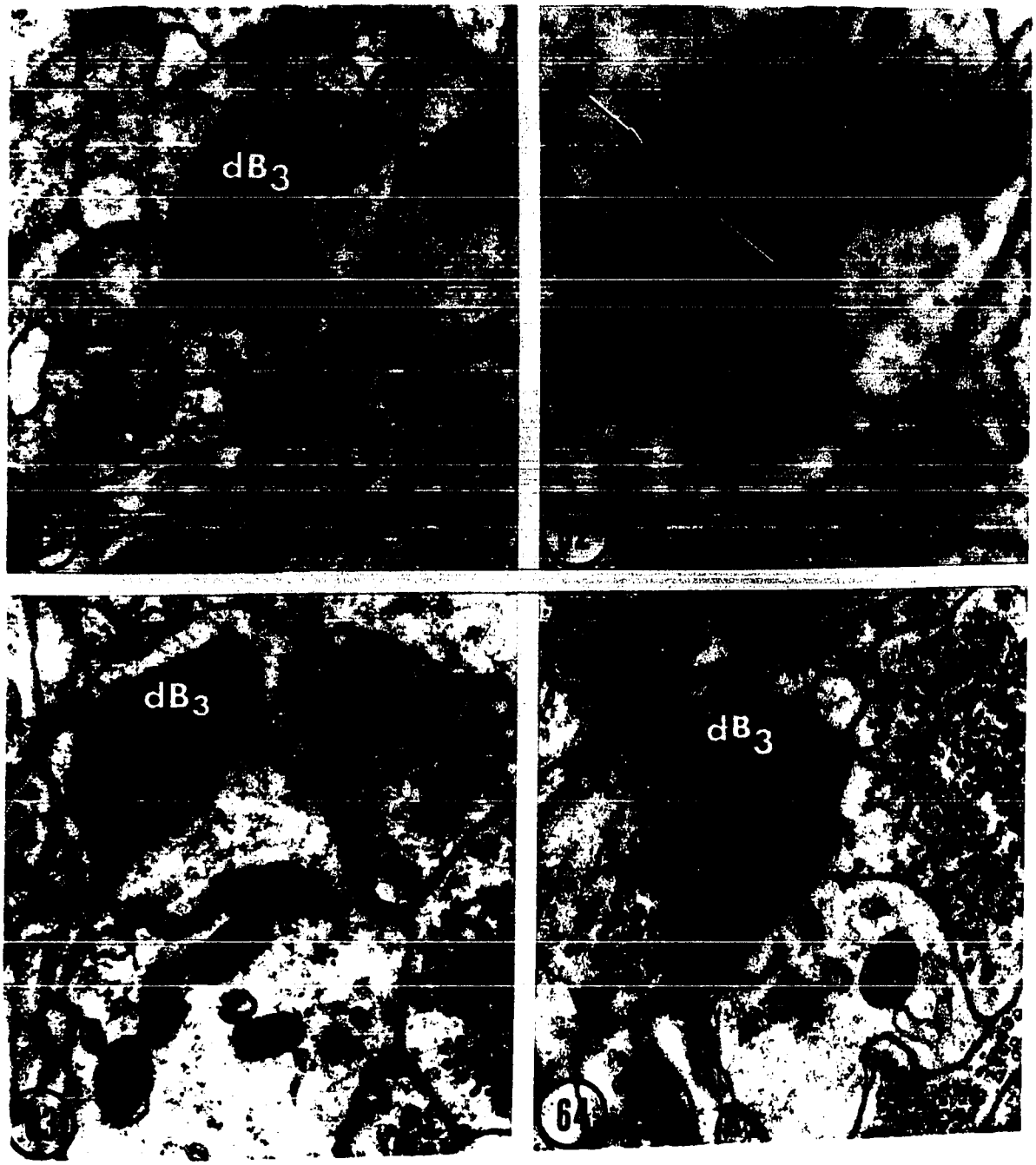
Electron micrographs of degenerated boutons in the corticomedial group of amygdaloid nuclei following a lesion of the lateral preoptic area.

Fig. 61. Degenerated bouton in the medial division of the central nucleus. Cat EEM 3, 5 days survival.
Magnification: 35,000X

Fig. 62. Same degenerated bouton as seen in figure 61 but taken at a higher magnification. Note mitochondrion between arrows.
Magnification: 70,000X

Fig. 63. Degenerated bouton in the medial nucleus. Cat EEM 4, 7 days survival.
Magnification: 35,000X

Fig. 64. Degenerated bouton in the lateral division of the central nucleus. Cat EEM 5, 15 days survival.
Magnification: 35,000X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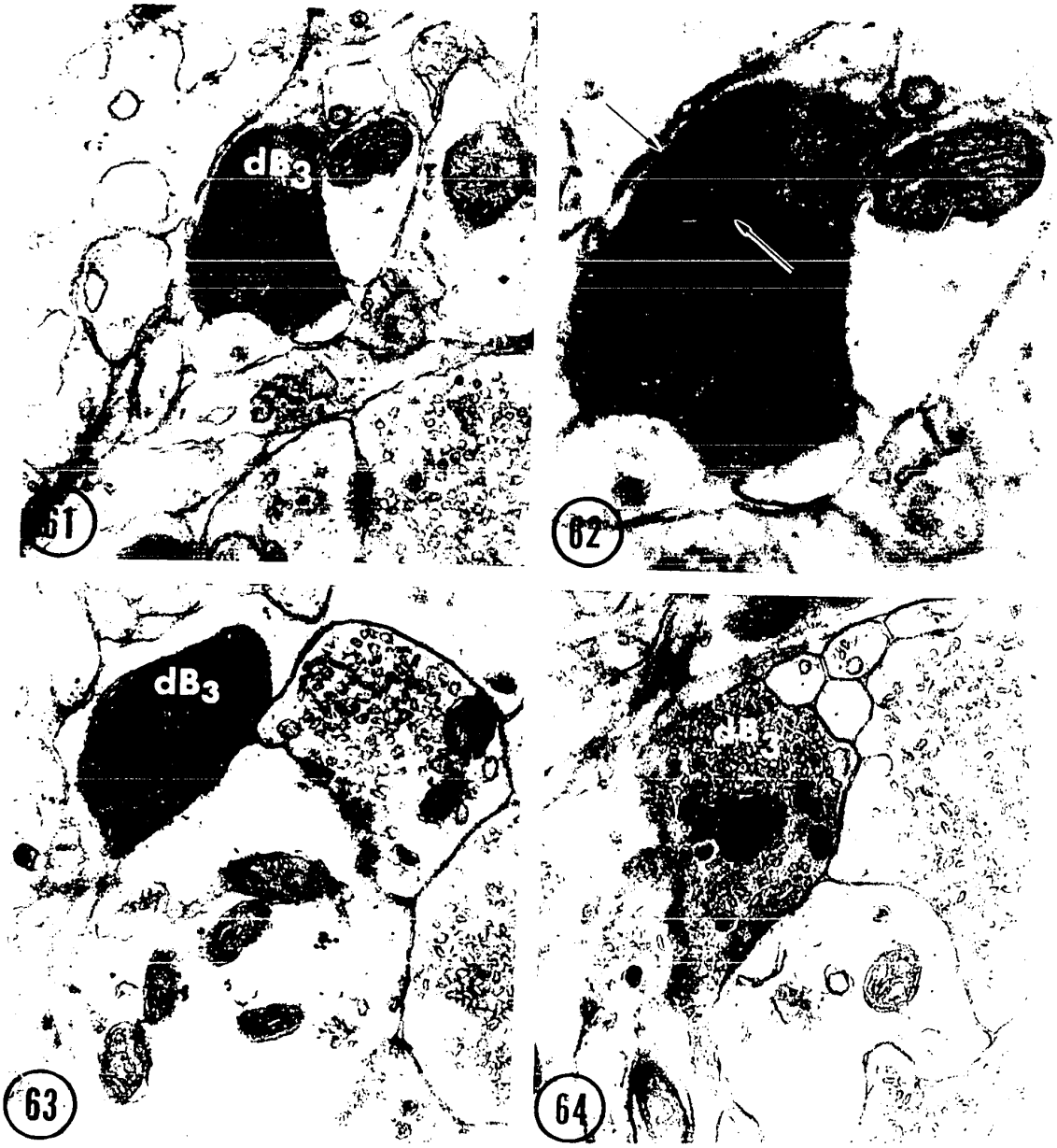
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Electron micrographs of degenerated boutons and axon in the basolateral group of amygdaloid nuclei following a lesion of the lateral preoptic area.

Fig. 65. Degenerated axon in the lateral nucleus. Cat EEM 3, 5 days survival.

Magnification: 35,000X

Fig. 66. Degenerated bouton in the large-celled part of the basal nucleus. Cat EEM 2, 3 days survival.

Magnification: 35,000X

Fig. 67. Degenerated bouton in the lateral nucleus. Cat EEM 3, 5 days survival.

Magnification: 35,000X

Fig. 68. Degenerated bouton in the small-celled part of the basal nucleus. Cat EEM 5, 15 days survival.

Magnification: 35,000X

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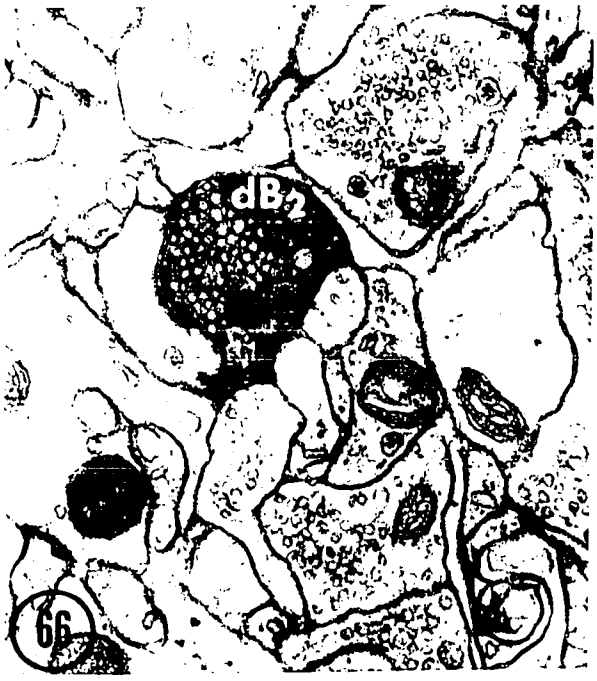

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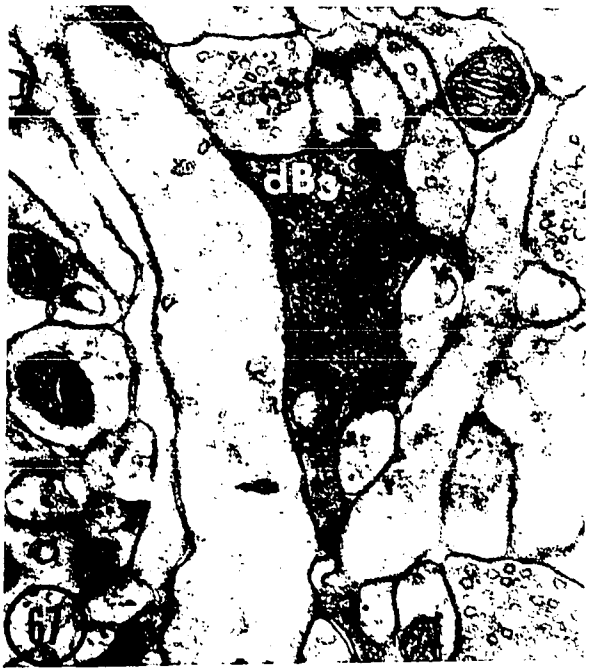

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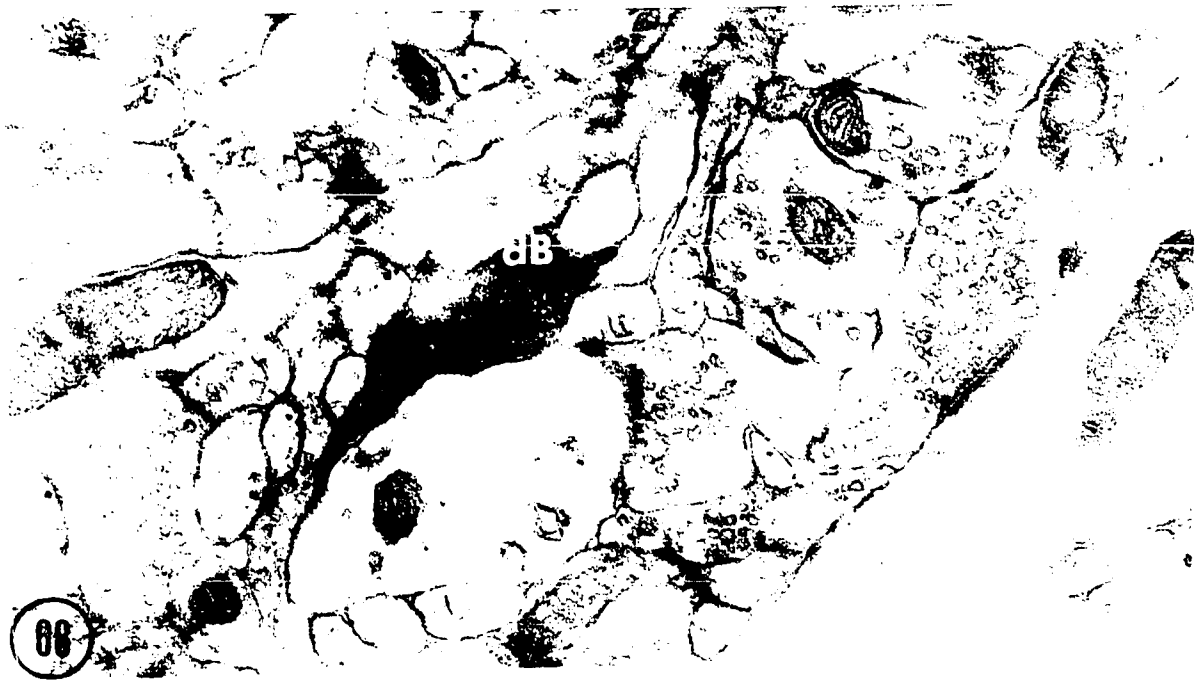

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CHAPTER IV

Discussion and Conclusions

The basic subdivisions of the amygdaloid nuclei as described in the cat by Fox (1940) were easily identified in the material stained by the cresyl echt violet or toluidine blue methods. The large-celled basal nucleus, with its large and deeply staining cells, is the most prominent nucleus of the amygdala.

The cortical, medial and central nuclei were described by Johnston (1923) as constituting the phylogenetically older nuclei of the amygdala. The central nucleus can be subdivided on the basis of cell size, in agreement with the observations of Fox (1940). As stated by this author, a large-celled division lies medial to a small-celled lateral division. The cells of the lateral portion are similar to those of the putamen. However, in an illustration of the amygdala in the cat, Koikegami (1963) has located the small-celled portion of the central nucleus in a position ventral to the large-celled part. In the present investigation the area ventral to the medial portion of the central nucleus is occupied by a group of small,

closely packed cells identified as one of the intercalated masses. The morphological appearance of this mass of cells is identical to a more lateral group, also identified as an intercalated mass, in agreement with Fox, (1940).

Because of the similarity in cell type between the lateral part of the central nucleus and the putamen, Fox (1940) suggested that the lateral part of the central nucleus be referred to as the putamen-central nucleus. This terminology leaves some uncertainty as to whether the lateral group of small cells represents a subdivision of the central amygdaloid nucleus, as first suggested by Johnston (1923), or a transitional area between the central nucleus and the putamen.

It was noted in the Nissl material of the present study that the lateral subdivision of the central nucleus contains a homogeneous population of small cells. In contrast, the putamen contains a number of large deeply stained cells scattered among the smaller cells. The presence of two cell types in the putamen of the cat has also been noted in material prepared for light microscopy by Adinolfi (1971) and in electron microscopic material of the putamen in the rat by Mori (1966). The presence of only small cells

in the lateral part of the central nucleus suggests that it was correctly identified as a subdivision of the central nucleus.

The electron microscopic observations of the normal central nucleus and the putamen lend support to this interpretation. It was noted in the electron microscopic study of the normal putamen that the majority of the boutons contained round synaptic vesicles and terminated on dendritic spines or small dendritic trunks. However, more than half of the boutons in both the lateral and medial regions of the central nucleus contain flattened vesicles and form axodendritic contacts. This difference in bouton population suggests that the afferent input to the lateral area of small cells is more similar to the large-celled central nucleus than to the putamen.

The putamen is known to receive afferent fibres from the thalamus, cerebral cortex and the substantia nigra (as mentioned by Mori, 1966). The afferent fibres

to the small-celled part of the central nucleus are not well known. The experimental results of the present study indicate that the more medial part of this subdivision receives a sparse projection from the hypothalamus. The more lateral segment receives fibres from the temporal cortex (Druga, 1969; Lescault, 1969, 1971). This would suggest that only the more lateral extreme of the small-celled central nucleus might possibly belong with the putamen. Since this segment cannot be distinguished from the putamen under the dissecting microscope, it was not included in the electron microscopic observations. Therefore, the ultrastructural characteristics of this area are not known. It would be interesting to know if the majority of the boutons contain round vesicles as in the putamen.

No differences were found in the fine structure of the cells in the two subdivisions of the central nucleus. A large portion of the soma in both the medial and lateral divisions was covered by glia. However, the extensive layering of glial processes as described around the soma in the medial amygdaloid nucleus by Hall (1968) was not seen in the central nucleus. One unique feature of the soma in both subdivisions of the central nucleus which has not been reported in any of the other amygdaloid

nuclei is the appearance of spine-like projections. The projections do not contain a spine apparatus and usually do not have a bulbous ending. A similar type of somatic projection has been described in the cuneate nucleus by Walberg (1966); in the lateral vestibular nucleus by Mugnaini et al. (1967) and in the lateral cervical nucleus by Westman (1968).

The electron microscopic features of the boutons in the medial large-celled portion of the central nucleus are very similar to those in the lateral subdivision. Synaptic boutons containing flattened vesicles (type 3) are a prominent feature of the central nucleus and account for a large number of the total bouton population. According to Hall, boutons with round vesicles (type 1) are the most common in the medial and lateral nuclei, (Hall, 1968) and in the cortical nucleus (Hall and Prym, 1972). Therefore, when compared with the medial, cortical and lateral amygdaloid nuclei, the central nucleus may be distinguished by the predominance of type 3 boutons.

A classification of the boutons based on vesicle population has been done in other parts of the basal ganglia. In material perfused with 4% glutaraldehyde fixative buffered with 0.1M cacodylate, Mori (1966)

identified five vesicle types in the corpus striatum of the rat. Terminals with small, round vesicles and terminals with large, round vesicles predominate. These small and large vesicles may have fine dense granules on their membranes (α - terminals and β - terminals, respectively) as in the neostriatum or the dense granules may be lacking (γ - terminals and δ - terminals) as in the globus pallidus. Granular vesicles were seen intermingled with other kinds of vesicles in the globus pallidus.

The present observations made in formalin fixed material do not agree with the observations by Mori (1966). No dense vesicles were identified in any of the boutons in the putamen. Although most of the vesicles were round, in agreement with Mori, a few boutons with flattened vesicles were also observed. The differences in morphological appearance of the vesicles in the two studies might be accounted for by the differences in fixative or in the buffer used. The primary fixative used by Mori was glutaraldehyde although he mentions that the dense granules on the membranes of the vesicles in the neostriatum were best seen in material which had been post-fixed in Dalton's fixative. The failure to find flattened vesicles may be accounted for by the osmolarity of the cacodylate buffer. Valdivia (1971)

has demonstrated a close relationship between osmolarity of phosphate and sodium cacodylate buffers and the vesicle shape in cerebellar tissue fixed in aldehydes. This author stated that when the osmolarity of the buffer was between 200 and 800 mOs the ratio of the flat and round vesicles was not changed. Below 200 mOs the number of round vesicles was increased, above 800 mOs the number of flats was increased. He also noted that this change was dependant on the osmolarity of the buffer and did not depend on the concentration of the aldehydes or on the total osmolarity of the fixative. Unfortunately, Mori did not give the osmolarity of the cacodylate buffer he used.

Recently, Adinolfi (1971) investigated the synaptic junctions in the cat putamen perfused with either phosphate-buffered 2% glutaraldehyde and 2% paraformaldehyde, or phosphate-buffered 5% glutaraldehyde. This author reported the presence of both round and flattened vesicles in the putamen. The majority of the presynaptic profiles contained round vesicles and were in synaptic contact with dendritic spines. The observations of the normal putamen in the present investigation agree with these findings.

Adinolfi classified the boutons in the putamen as type 1 or type 2 synapses.

These types are similar to the type 1 and 2 of Gray (1959).[2] However, it has been noted by Colonnier (1968) that the width and structure of the intersynaptic cleft are not the same in formalin fixed material as in the osmium immersed material of Gray. Colonnier has introduced a more descriptive terminology in which the synapses with a postsynaptic density are referred to as asymmetrical synapses, the others as symmetrical synapses. This author found that in the cerebral cortex the asymmetrical synapse is usually associated with the presence of round synaptic vesicles in the presynaptic profile while the symmetrical synapses are associated with the flattened type of synaptic vesicle. In the present electron microscopic investigation of the central amygdaloid nucleus and the putamen, the morphology of the synaptic complex is similar to that described by Colonnier. However, the association of round vesicles with an asymmetrical synaptic complex was not always true of the axosomatic synapses. In some of the axosomatic contacts in the central nucleus and the putamen, the postsynaptic density usually associated with round vesicles was not always obvious.

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2 Gray's type 1 has, as compared to type 2, a thicker postsynaptic membrane specialization, a wider intersynaptic cleft and a longer region of synaptic contact.

Experimental Studies

The experimental studies have confirmed previous reports of amygdalopetal projections from the hypothalamus. Following selective lesions of specific hypothalamic areas, only the anterior hypothalamic nucleus and the lateral preoptic area were found to project to the amygdaloid complex.

The fibres from the anterior hypothalamic nucleus course through the lateral hypothalamus to join the ventral pathway to the amygdala. Very sparse degeneration was seen in the central, basal and lateral nuclei. Cowan et al. (1965) reported degenerated terminal fibres in all of the amygdaloid nuclei, with the exception of the central nucleus, after a lesion in the hypothalamus just lateral to the anterior hypothalamic nucleus. This portion of the rostral lateral hypothalamus was not lesioned in the present series of experiments. However, fibres from the anterior hypothalamic nucleus pass through the lateral hypothalamus and would have been interrupted by the lesion in the experiments of Cowan et al. Therefore, it is likely that some of the degeneration reported by these authors came from the anterior hypothalamic nucleus.

The distribution of degeneration in the amygdala

after a lateral preoptic lesion is similar to that reported by Nauta (1958). Degenerated fibres from the lateral preoptic area course in both the stria terminalis and ventral pathway to terminate in all of the amygdaloid nuclei, with the exception of the cortical nucleus. Although Nauta did not describe degenerated fibres in the lateral nucleus, this projection is not a heavy one and could easily be overlooked. No degeneration could be followed to the cortical nucleus in these experiments or in the study by Nauta. Such a projection was identified in the rat by Cowan et al. (1965) and may indicate a species difference.

In the course of staining each brain by one of the methods for the impregnation of degenerated axons and their terminals, the degenerated fibres were easily demonstrated. However, some difficulty was encountered in determining the termination of these fibres within the amygdaloid nuclei. The hypothalamic afferents to the amygdala were very sparse. In most instances, a few fine fibres without specific orientation were identified as preterminal fibres in the material stained by the Nauta (1957) method. Other fibres seemed to end abruptly as small tear-shaped droplets. The droplets were interpreted as degenerated terminal endings.

The impregnation methods for the demonstration of degenerated axons and their terminals as developed by Nauta and Ryan (1952), and its modifications (Nauta and Gygax, 1954; Nauta, 1957), have been an important contribution to investigations of the course and termination of pathways in the central nervous system. These 'suppressive' Nauta methods have overcome the difficulty of the earlier techniques which stained normal and degenerated fibres and their terminals with equal intensity. However the use of the suppressive Nauta methods to demonstrate terminal degeneration has been questioned by some authors (Evans and Hamlyn, 1956; Bowsher et al. 1960; Heimer, 1967; Walberg, 1971).

It has been reported that the Fink and Heimer modification (1967) of the Nauta and Gygax (1954) method is more selective for staining the axon terminal than the suppressive Nauta methods (Heimer and Peters, 1968). The hypothalamic terminals in the amygdala, when stained by this method, appeared as small scattered silver granules. These terminals had the same distribution within the amygdaloid nuclei as the terminal fibres identified in the material impregnated by the Nauta (1957) method. However, identification of terminals in the material impregnated by the Fink and Heimer

method was frequently hampered by the appearance of small silver granules distributed evenly over the entire section. In these cases, a careful comparison of the number and distribution of the silver granules on the operated side with the contralateral side had to be made. Since the amount of degeneration to most of the amygdaloid nuclei was very sparse, this task usually proved impossible.

It was concluded that when the connections are very sparse, such as the hypothalamic afferents to the medial, basal, and lateral amygdaloid nuclei, the use of the electron microscope is necessary to confirm the presence of degenerated terminal boutons.

The early degenerative changes in the terminal boutons of the hypothalamic axons in the amygdala are similar to those described in the visual cortex (Colonnier and Gray, 1962; Colonnier, 1964), in the inferior olive (Walberg, 1964), in the hippocampus (Alksne et al., 1966), the vestibular afferents in Deiters' nucleus (Mugnaini et al., 1967) and in the prepiriform cortex (Westrum, 1969). These authors reported an increase in electron density and granulation of the axoplasm and an increase in density of the mitochondria.

Some authors reported that glial cells appeared to engulf the degenerating processes (Colonnier, 1964; Mugnaini et al., 1967). However, no glial engulfment was observed in the amygdaloid nuclei. Many times a process of an astrocyte was seen in close proximity to the degenerated bouton. However, glial processes were also observed in proximity to normal boutons so that it could not be determined if this was a phagocytic response in the experimental material.

Recently, Cohen and Pappas (1969) presented data from the ventrobasal thalamic nucleus in the cat which suggested that an increase in electron density might not be a reliable indication of early anterograde degeneration. These authors found electron dense boutons in apparently normal material.

However, in the present material, the boutons became progressively more electron dense and shrunken with increased post-operative survival times. These degenerated or dark boutons usually contained flattened vesicles even when the predominant type of vesicle was of the round variety.

Electron dense myelinated axons were frequently observed in all of the amygdaloid nuclei with the

exception of the cortical nucleus. In the normal material observed by Cohen and Pappas, no myelinated axons were electron dense.

It is concluded that the electron density of the profiles seen in the central, medial, basal and lateral amygdaloid nuclei after a lateral preoptic lesion is a sign of anterograde degeneration.

In some areas of the brain, filaments appear as a result of the degenerative process, as in the avian optic tectum (Gray and Hamlyn, 1962) or the filaments already present hypertrophy, as in the retino-geniculate terminals (Colonnier and Guillery, 1964 and Guillery, 1965; Szentagothai et al., 1966). This filamentous reaction may be followed by an increase in electron density of the bouton, as in the optic nerve fibres (Szentagothai et al., 1966) and in the Purkinje cell boutons in Deiters' nucleus (Mugnaini and Walberg, 1967). No filaments were observed in the normal or the degenerated boutons in the amygdala.

In the course of the experimental electron microscopic observations it became obvious that a large percentage of the boutons in the amygdala did not degenerate following a hypothalamic lesion. The question of the origin of these additional afferents is not

completely answered by previous investigations of amygdalopetal connections.

The known afferent projections are, in the case of the basal and lateral nuclei, from the temporal cortex (Lescault, 1969, 1971; Druga, 1969), from the piriform cortex (Valverde, 1965; Cowan et al., 1965), intra-amygdaloid connections (Valverde, 1965) and hypothalamic afferents. The possibility of thalamic connections with the lateral nucleus of the amygdala has not been completely explored. There is only one suggestion of a direct thalamic connection in the cat and this was found in the course of an investigation of thalamocortical connections (Graybiel, 1970). A possible additional source of afferent fibres to the basal and lateral nuclei is an inter-amygdaloid connection from the temporal limb of the anterior commissure. This connection was noted in a comparative anatomical survey of the connections of the amygdala by Ariëns Kappers et al. (1936). However, Fox and Schmitz (1943) were unable to verify this pathway in the cat following a lesion in the anterior commissure. Since these authors used the Marchi method, only myelinated fibres would have been stained. Therefore, the possibility that this inter-amygdaloid component consists of finely

myelinated fibres cannot be ruled out.

It has been shown that the cortical and the hypothalamic boutons terminating in the lateral nucleus have different morphological characteristics. Cortical afferents terminate primarily on dendritic spines and their terminal boutons contain round vesicles (Hall and Prym, 1971). The present investigation shows that the hypothalamic afferents terminate primarily on dendritic trunks and their terminal boutons contain flattened vesicles. It has been suggested by Uchizono (1965) that the terminals containing flattened vesicles are inhibitory whereas those containing round vesicles are excitatory. This suggests that the cortical and hypothalamic afferents have opposite electrophysiological effects on the postsynaptic dendrites in the lateral nucleus. In a study of evoked potentials in the lateral amygdaloid nucleus elicited by neocortical and hypothalamic stimulation, Caruthers et al. (1964) noted that the evoked responses mutually interact. Since this interaction resulted in attenuation of the test response (whether of cortical or hypothalamic origin), these authors suggest that the cortical and hypothalamic impulses are linked to cause inhibition in the lateral nucleus of the amygdala.

The afferent connections of the central, medial and cortical amygdaloid nuclei are not well known. The present studies indicate that the hypothalamus sends a moderate number of fibres to these nuclei. The boutons of the hypothalamic afferents contain flattened vesicles. Only olfactory tract fibres have been shown to terminate in the cortical nucleus (Scalia, 1968). There is some question as to whether the central and medial nuclei receive fibres from the lateral olfactory tract. An electron microscopic study of the olfactory tract terminations in the amygdaloid complex would be a valuable aid in clarifying this point.

In a discussion of the interconnections of the limbic system, Nauta (1958) has noted various pathways through which the hippocampus and amygdala can project to the mid-brain. He noted that both the hippocampus and the amygdala project to the lateral preoptic area. The lateral preoptic area projects through the medial forebrain bundle to terminate in central and lateral regions of the mid-brain tegmentum. Nauta refers to this mid-brain region as the 'limbic' mid-brain region. Ascending connections from the limbic mid-brain area "close a limbic system-mid-brain circuit"[3].

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3 Nauta, W.J.H., 1958, page 339

The ascending projections from the anterior hypothalamus to the amygdala appear to be the last link in the ascending portion of a limbic system-mid-brain circuit which terminates in the amygdala.

No degeneration to the amygdala was detectable after any of the lesions located caudal to the anterior hypothalamus. Since degenerated fibres to other areas of the brain were well impregnated, the lack of degeneration to the amygdala cannot be attributed to technical problems related to staining.

The degeneration reported after lesions in the lateral hypothalamus is consistent with the findings of Nauta (1958) and Umemoto (1968).

Following lesions of the ventromedial hypothalamic nucleus in cats, Kaelber and Leeson (1967) traced degenerated fibres into the dorsal hypothalamic nucleus, the posterior hypothalamic nucleus, the anterior hypothalamic nucleus and rostrodorsally to the nucleus paraventricularis. The survival times were varied from 7 to 21 days with no difference in the distribution of the degeneration being noted. In the present investigation, the same pattern of degeneration was observed 8 days after a lesion of the ventromedial nucleus. The projection to the dorsomedial nucleus of the thalamus as mentioned by Johnson (1965) was not

observed in these studies or in those of Kaelber and Leeson. This difference in results cannot be accounted for by differences in survival time or staining methods.

The present investigation has shown that the hypothalamic afferents in the amygdaloid nuclei can be identified by the morphological appearance of the synaptic ending and its site of termination. The terminal boutons of the hypothalamic afferents contact dendritic trunks and contain flattened vesicles.

In a previous investigation of the morphology of the cortical afferents in the lateral amygdaloid nucleus, Hall and Prym (1971) found that the terminal boutons contained round vesicles and terminated on dendritic spines.

The implications of these findings for future investigations of afferent systems within the amygdaloid complex are promising. Of particular interest are the olfactory tract connections since there is some question as to their exact nuclei of termination. An electron microscopic study of the areas of terminal degeneration following olfactory tract lesions would allow identification of degenerated terminal boutons and their site of termination on the postsynaptic neuron.

CHAPTER V

Summary

1. The nuclear subdivisions of the amygdala in the cat were studied in Nissl preparations.
2. The fine structure of the central amygdaloid nucleus and the putamen, as observed with the electron microscope, was described.
3. It was noted that more than half of the boutons within the central nucleus contain flattened vesicles.
4. The boutons in the putamen contain primarily round vesicles.
5. The lateral division of the central nucleus, which is difficult to delimit from the putamen in Nissl preparations, was distinguished from the putamen in electron microscopic material by its large number of boutons containing flattened vesicles.
6. Small lesions were placed at various rostro-caudal levels of the hypothalamus. The animals were allowed to survive for 4, 8, or 11 days. The cat was then perfused through the heart

with 10% formalin and the brain removed from the skull. The brain was fixed further in 10% formalin for six weeks prior to staining.

7. The brain was sectioned at a thickness of thirty microns and stained by the Nauta (1957), Fink and Heimer (1967) or Wiitanen (1969) methods.

8. It was observed that the anterior hypothalamic nucleus projects to both subdivisions of the central nucleus, both divisions of the basal nucleus and the lateral nucleus of the amygdala by way of the ventral pathway.

9. The lateral preoptic area projects to the anterior amygdaloid area, both divisions of the central, both divisions of the basal, the medial and the lateral amygdaloid nuclei by way of the stria terminalis and the ventral pathway.

10. The morphology of the terminal boutons in the central, medial, basal and lateral amygdaloid nuclei was described 2, 3, 5, 7, and 15 days after a lesion of the lateral preoptic area.

11. The degenerated boutons appeared electron dense and became progressively shrunken with

longer post-operative survival times. The vesicles, when distinguishable, were nearly always of the flattened variety in all of the nuclei.

12. The potential value of the electron microscopic findings for identifying different afferent systems within the amygdaloid complex was discussed.

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