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CONNECTIONS OF THE VISUAL CORTEX WITH THE CLAUSTRUM

Dymitris CHADZYPANAGIOTIS and Olgierd NARKIEWICZ

Department of Anatomy, School of Medicine,
Gdańsk, Poland

The function of the claustrum, one of the main nuclei of the telencephalon is virtually unknown. Some light, however, has been thrown on its role in the central nervous system by recent investigations on telencephalic connections. These have shown (Narkiewicz 1964, 1966, Carman et al. 1964, Druga 1966*a*) that the claustrum is connected with almost all the areas of the neocortex. There is also a topographical relation of very definite regions of the claustrum with appropriate cortical areas. These connections are so numerous that the removal of any given area of the neocortex leads to the degeneration of the claustral cells.

The general scheme of the connections of the cortex with the claustrum has been investigated (Narkiewicz 1964) in the cat. Their essential arrangement has also been studied in the rabbit (Carman et al. 1964). On the basis of the above mentioned studies and our own unpublished observations on other animals it seems that the organization of the cortical connections with the claustrum is similar in various animals. This allows the assumption that our experimental results are also true for other species of mammals including man.

There are no detailed investigations concerning the connections of the claustrum with cortical projection areas, i.e. visual, auditory and sensory. An exact knowledge of these is of essential significance for the further development of electrophysiological studies on the claustrum and will facilitate the determination of the relations between the claustrum and the system conveying impulses to the cortex.

The aim of our work is to study the connections of the visual cortex

and especially (i) to ascertain which parts of the claustrum possess connections with the visual cortex and (ii) to determine the probable direction of these connections, whether they are axons of the cortical neurons conveying impulses from the visual cortex to the claustrum or axons of claustral cells running to the cortex. This problem which is essential to an understanding of the role of the claustrum in the central nervous system has not as yet been solved. The majority of investigations based on methods of the Wallerian degeneration showed the existence of the corticoclaustral connections. But the occurrence of severe degenerative changes in the claustral cells leads to the assumption that the claustrocortical axons may possibly be present also in this area.

MATERIAL AND METHODS

Degeneration methods were used in our experimental studies. After removal of the visual cortex degeneration of the neurons, axons and synaptic boutons was studied. Our investigations were carried out on cats in which the structure of the claustrum is relatively well known.

In the experiments 30 adult cats, weighing 1500–4500 g were used. All the animals underwent unilateral surgical treatment, usually with the removal of the cortex from the left hemisphere. The extent of the removal concerned the visual cortex or the visual cortex together with the neighbouring areas. Surgery was carried out with adherence to aseptic principles in general anesthesia, injecting Nembutal intramuscularly in doses of 40 mg/kg of body weight. The operative field was sterilized and a trepanation performed over the visual cortex. The dura mater was cut and drawn apart. The bared cerebral cortex was removed by suction.

The period of survival ranged from 3 to 76 days. The animals were sacrificed in general anesthesia produced by intraperitoneal application of Nembutal. Perfusion was carried out by means of a 0.9% NaCl solution given at about 60 drops per minute. Further perfusion was performed with a 10% formalin solution. The brain thus fixed was removed as a whole and preserved in a 10% solution of formalin.

To examine the degeneration of the nerve cells the specimens were stained with cresyl violet, the silver nitrate methods were used to reveal the degeneration of axons and synaptic boutons, for electron microscopic examinations the material was fixed in osmium tetroxide.

The experimental material was divided as follows into 4 groups according to the staining method and survival period:

Group	Staining method	Survival period (in days)	Number of animals
1	Cresyl violet (Nissl)	35-76	18
2	Cresyl violet (Nissl)	5-25	4
3	Nauta and Fink–Heimer	5-6	6
4	Osmium tetroxide (specimens for electron microscopy)	3-5	2

The brains for staining with cresyl violet (Groups 1 and 2) were embedded in paraffin blocks and cut into serial sections, 15–20 μm thick.

The brains of Group 3 were prepared by means of the Nauta method (Nauta 1957) and Fink–Heimer II modification (Fink and Heimer 1967). Sections about 20 μm thick were cut and impregnated with silver nitrate. For electron microscopic examinations (Group 4) the dorsocaudal portion of the claustrum of the cat was removed. The material was fixed at temperature of 4°C during 1 hr in glutaraldehyde mixed with osmium tetroxide, the ratio of glutaraldehyde to osmium being 1:1 (according to Franke) in 0.1 M cacodylate buffer plus saccharose, washed during 15 min, then fixed during 1 hr in a 2% solution of osmium tetroxide with the same buffer plus saccharose. Dehydration in a gradually increasing concentration of alcohol and acetone was followed by embedding in epon 812. The sections were cut with a glass knife on Reichert microtome OM-U2, additionally stained with lead citrate and uranil acetate, then observed under a JEM 7A microscope.

The location of the cortex removed was determined by projecting every 20th specimen on milimeter paper, followed by reconstruction.

The extent and degree of degeneration in the claustrum were determined in all the animals investigated. The results obtained were shown on diagrams of cross-sections through the posterior portion of the claustrum. Besides, a table was made for each animal including the following data: reconstruction of the damaged area of the cerebral cortex, diagrams of cross-sections of cerebral hemispheres with the extent of removal and a series of diagrams of cross-sections of the claustrum with superimposed areas of degeneration.

THE STRUCTURE OF THE CLAUSTRUM

The claustrum is a structure appearing in all mammals. It is situated under the cortex on the borderline of the paleo- and neocortex.

Among the studies concerning the structure of the claustrum in man the most noteworthy are those of Pintus (1930, 1931, 1932), Brockhaus (1940), Landau (1936, 1938) and Stelmasiak (1955). Moreover Rae (1954*ab*)

compiled the results of previous investigations on the structure and topography of the human claustrum and showed the differences existing in this field between particular authors.

There is a relatively small number of studies concerning the structure of the claustrum in animals. The claustrum of the rabbit has been described by Rose (1928) and Young (1936); of the rat — Gurdjian (1928); of the bat — Humphrey (1936); of the opossum and beaver — Pilleri (1961, 1962); of the dog — Tenerowicz (1960). Studies on the claustrum of the cat are somewhat more numerous (Druga 1966*b*, Narkiewicz 1964, Berlucchi 1927). De Vries (1910) while studying the origin of the claustrum also made a comparative survey of its structure in various animals and in man.

In the cat the claustrum is a structure generally well separated from its surroundings, extending 12–14 mm orocaudally, 1.5–2 mm mediolaterally and 9–11 mm dorsoventrally. The width of the claustrum is much smaller than its length, in spite of the fact that it varies greatly in particular sections.

Within the claustrum two fundamental parts can be differentiated — the dorsal and the ventral one. The transition between them occurs just at the bottom of the rhinal sulcus. The dorsal part, phylogenetically more recent, called the claustrum insulare, lies under the insular cortex. The ventral part, the claustrum prepiriforme strongly developed in lower mammals is located under the prepiriform cortex.

The claustrum insulare. The insular claustrum is located between the putamen and upper part of the amygdaloid complex from the medial side and insular cortex from the lateral. A very distinct external capsule separates the insular claustrum from the putamen and amygdala. On the other side the extreme capsule located between the claustrum and the insular cortex includes a great number of large cells similar to the claustral cells or to those from the deepest cortical layers.

In its frontal portion the insular claustrum appears as a narrow strip of cells the upper pole of which widens clubwise. The intermediate portion has the shape of a triangle in its frontal cross-section. The caudal portion (Fig. 10) is elongated dorsoventrally, gradually narrowing downwards; at the level of the rhinal sulcus it fuses with the prepiriform claustrum.

The claustrum prepiriforme. The prepiriform claustrum is situated wholly below the rhinal sulcus (Fig. 10). In its rostral section the prepiriform claustrum appears as a small group of cells which fuse laterally with the deeper layers of the loosely arranged prepiriform cortex. Caudally the prepiriform claustrum gradually becomes larger, attaining its maximum dimensions in its intermediate portion.

In specimens stained by means of the Nissl method (Fig. 11–14) a great variety of cellular forms was found to occur in the entire claustrum, particularly in the claustrum insulare. These were mainly multipolar and round cells and a smaller number of pyramidal and spindle-shaped cells.

1. Multipolar cells (Fig. 11) — of various shapes with a centrally placed large, round, bright nucleus and nucleolus stain intensely. The cytoplasm encases the nucleus in a wide band and number of Nissl granules are generally found here. This type of cell is most numerous.

2. Pyramidal cells (Fig. 12) — are less numerous and appear most frequently singly. The egg-shaped nucleus stands out clearly from the well stained cytoplasm by which it is surrounded on all sides. The nucleolus stains intensely.

3. Round cells (Fig. 13) — are slightly smaller, enclose a bright, round nucleus taking up most of the cell. They are surrounded by a band of cytoplasm containing fine Nissl granules. As in multipolar and pyramidal cells the nucleolus is conspicuously separated.

4. Spindle-shaped cells (Fig. 14) — are found in the claustrum mainly from the side of the extreme capsule. The nuclei are elongated like the cells themselves. The cytoplasm contains fine Nissl granules. The nucleolus here is also dark and well separated as in the other types of cells.

RESULTS

Degeneration of the claustral cells following ablations of the visual cortex

The experimental material on which cellular degeneration was examined comprised 22 cats with various periods of survival and extent of ablations (Groups 1 and 2). In analyzing this material particular attention was paid to the location of the changes in the claustrum as well as to the intensity of degeneration in relation to time of survival.

Localization of the claustrum degeneration. The material presented in Fig. 1 permits a sufficiently exact determination of the degeneration localization in the claustrum. These are animals in which the greater part of the visual cortex was removed without destroying the neighbouring cortical areas. All the animals in this group showed degenerations in the same area of the claustrum, that is why only two cases will be described in detail (67-15 and 67-79).

In cat 67-15 (Fig. 2) the ablation includes large areas of the visual cortex on the medial surface of the hemisphere, within the splenial and suprasplenial gyrus. On the dorsal surface the greater parts of the lateral and posterolateral gyrus were removed. The suprasylvian gyrus is un-

touched. Altogether the greater part of the visual cortex was removed, both the striate as well as the extrastriate.

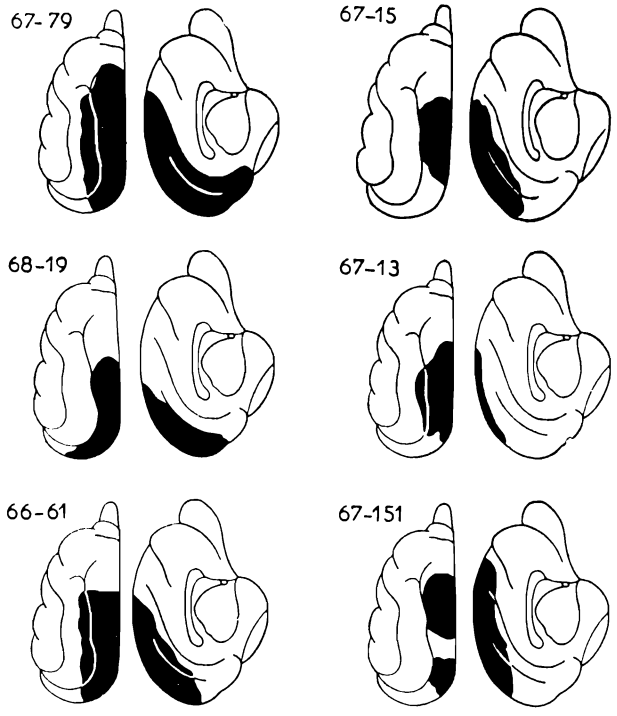

Fig. 1. The extent of cortical removals in cats 67-79, 68-19, 66-61, 67-15, 67-13 and 67-151 plotted on standard sketches of the dorsal and medial aspects of the brain. Other denotations as in Fig. 2.

In frontal cross-sections of the claustrum intense degenerative changes were found to be located in the dorsal part of its caudal portion.

In cat 67–79 (Fig. 3) the removed part of the cortex is slightly larger. On the dorsal surface it includes almost all of the lateral gyrus and the major part of the posterolateral gyrus as well as the medial areas of the suprasylvian gyrus. On the medial surface both the splenial and suprasplenial gyrus were completely removed. Thus, in this case the striate visual cortex was removed together with the greater part of the extrastriate cortex entering to a certain degree the cortex of the suprasylvian gyrus.

Degenerative changes in the claustrum (Fig. 15, 16, 17 and 18) are present only in the dorsocaudal part and in this case there are no great differences concerning size and severity of degeneration in comparison to the previous cats (67-15).

The two cases above as well as the others shown in Fig. 1 prove that the visual cortex possesses connections with the claustrum. It is connected with its dorsal part situated in the caudal portion. In frontal cross-sections this area includes the upper pole of the claustrum. Despite the

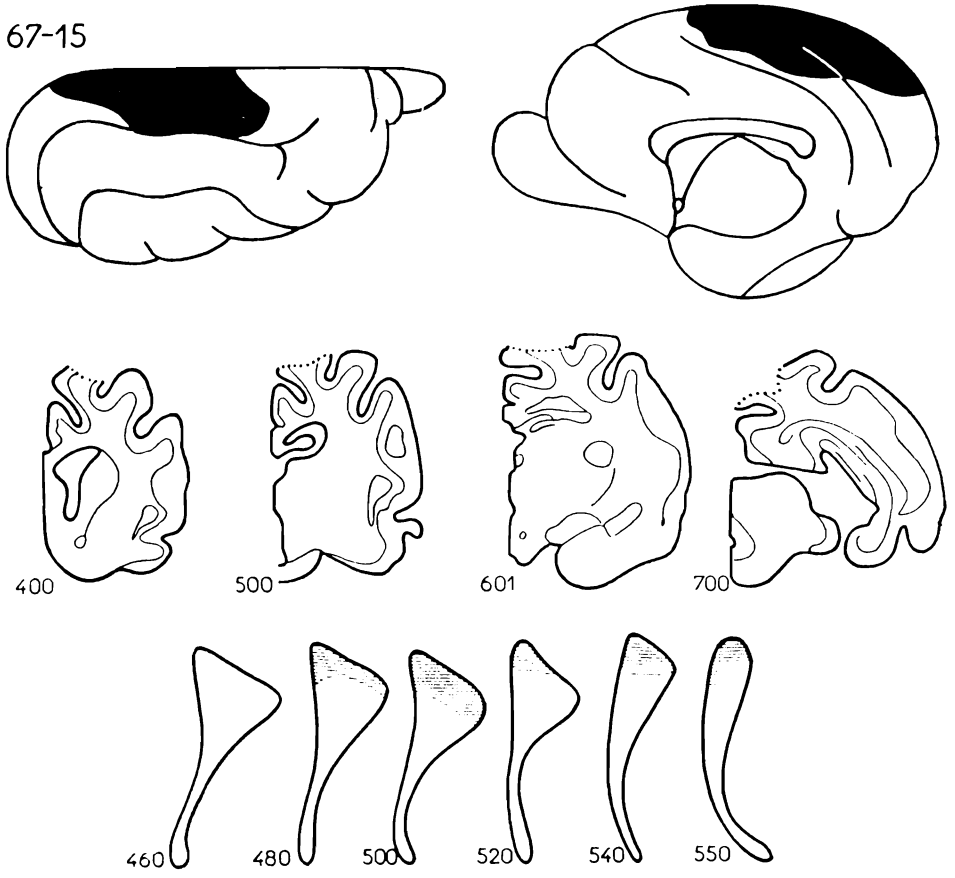

Fig. 2. Diagrams showing the extent and depth of the cortical lesion in cat 67–15 with the resulting degeneration of the neurons in the claustrum. Upper row, surface reconstruction of the removal plotted on standard sketches of the brain; intermediate row, cross-sections through the hemisphere; lower row, the destribution of the degenerations in the caudal portion of the claustrum indicated by horizontal stripes.

fact that the part of the claustrum connected with the visual cortex has a separate localization there is a gradual transition of the degeneration area into one in which there are no changed neurons.

In six cats the neighbouring areas of the cortex were removed in addition to the visual cortex. Three cases in this group will be described in detail (67–73, 67–66, and 67–99).

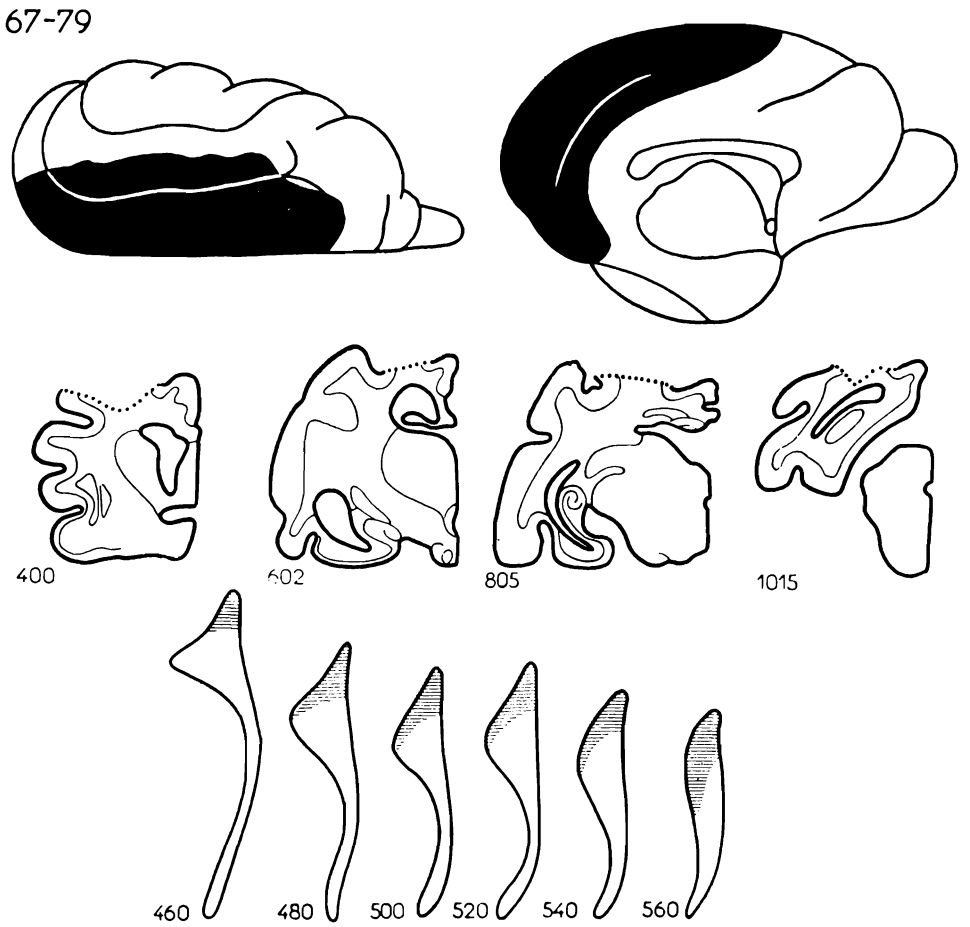

Fig. 3. Diagrams showing the extent and depth of lesions of the cortex in cat 67-79, with the resulting degeneration of the neurons in the claustrum. Other denotations as in Fig. 2.

In cat 67–79 (Fig. 3) the removed area on the dorsal surface of the hemisphere is limited mainly to the visual cortex of the lateral and posterolateral gyrus as well as to a small part of the suprasylvian gyrus. But on the medial surface, the splenial and suprasplenial gyrus and the caudal parts of the cingular gyrus were removed in addition to the visual cortex. The area of the cortex removed reaches the caudal part of the corpus callosum, including the visual and the limbic posterior cortex.

The area of the degeneration in the claustrum and the intensity of these changes do not differ much from those observed in the previous cases where the ablation concerned only the visual cortex. Thus it may

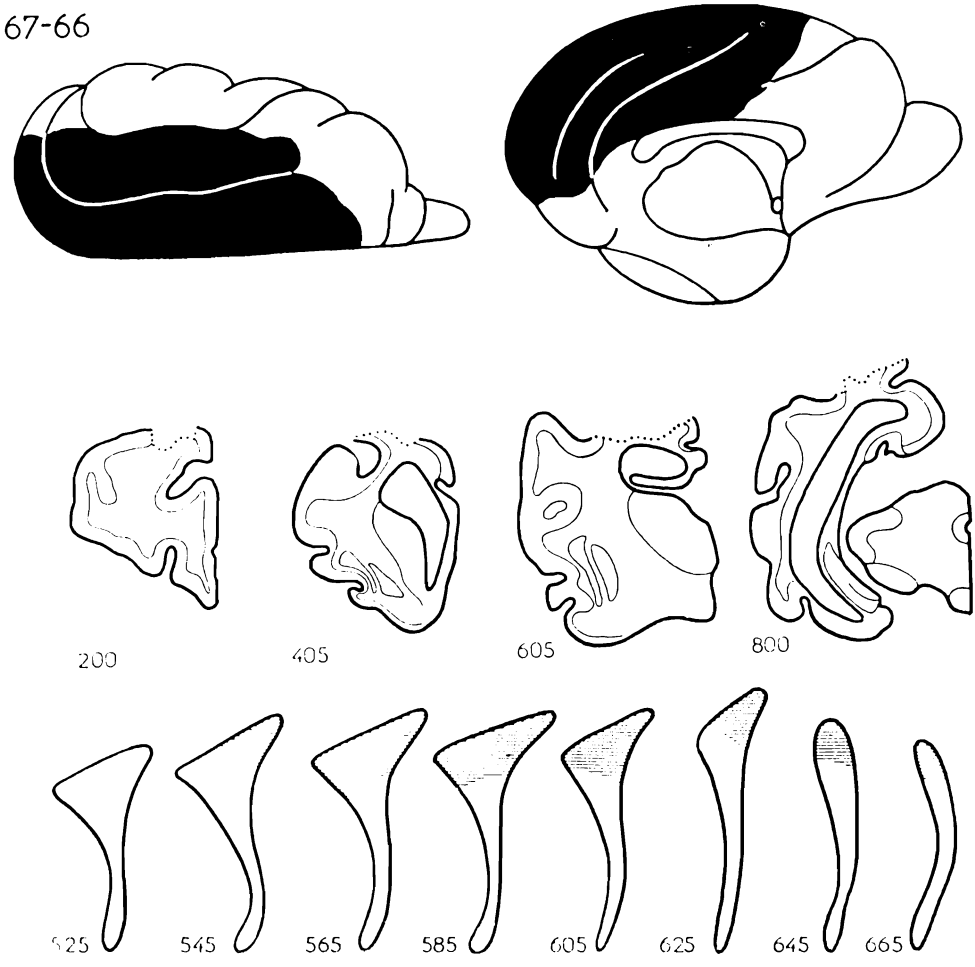

Fig. 4. Diagrams showing the extent and depth of lesion of the cortex in cat 67–66. Other denotations as in Fig. 2.

be assumed that the ablation of the limbic cortex does not cause discernible degeneration in the claustrum. Probably this cortex has no connections with the claustrum or if they do exist they were scantier than those of the visual cortex.

In cat 67–66 (Fig. 4) the ablation on the surface of the medial cortex was similar in size to that in the previous case; it included the splenial

gyrus, suprasplenial gyrus and posterior limbic cortex. But on the dorsal surface of the hemisphere the ablation reached much farther laterally than in the previous cases, including to a great degree the suprasylvian gyrus besides the lateral and the posterolateral gyrus. Degeneration is found to be similar to that in the previous case, in the caudal zone of the claustrum but it extends much lower and is also more severe.

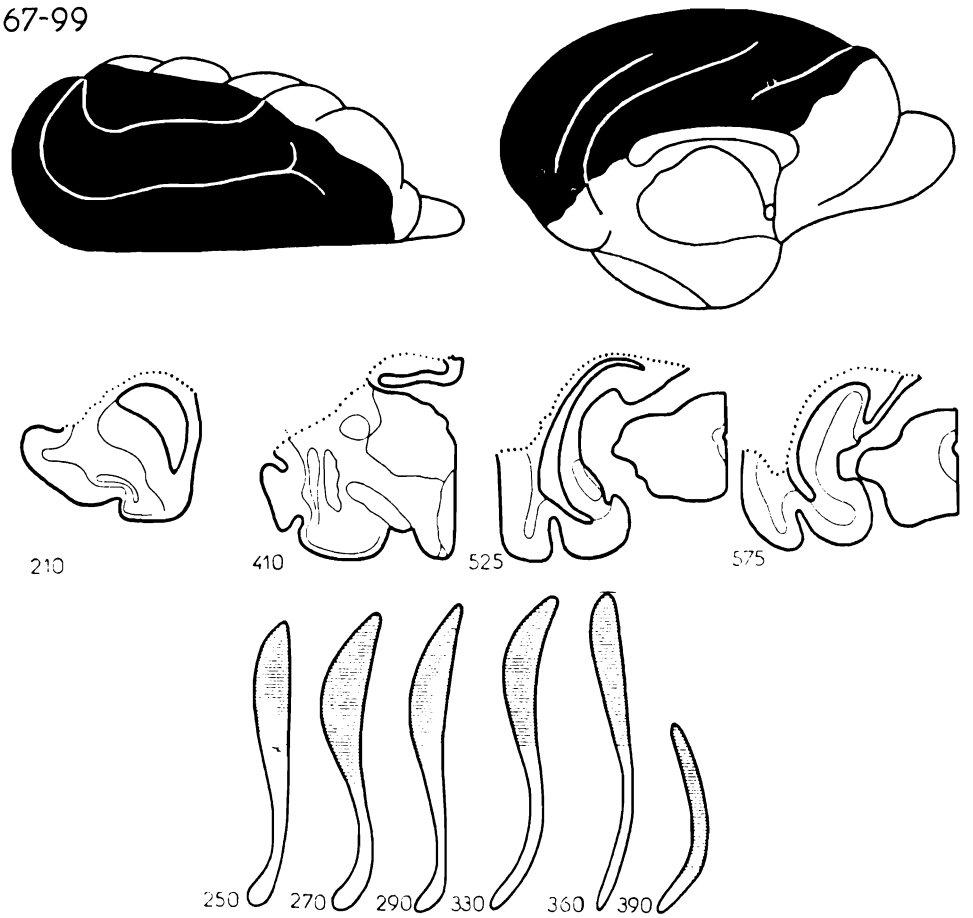

Fig. 5. Diagrams showing the extent and depth of lesion of the cortex in cat 67-99, with the resulting degeneration of the neurons in the claustrum. Other denotations as in Fig. 2.

In cat 67-99 the area of cortical ablation is still more extensive. Besides the posterior limbic cortex and visual cortex as well as the cortex of the suprasylvian gyrus, a major part of the auditory cortex and a small area of the sensory and motor cortex was removed.

A major cortical ablation caused severe changes in the claustrum. The borderline of the degenerative area shifted more frontally. Above all, however, a severe degeneration was found to occur in the caudal zone of the claustrum where it extended very far downwards. In comparison to the opposite side there is a distinct shrinking and deformation of the whole caudal portion of the claustrum.

These experiments showed decisively that an increase of the ablation so that it includes the more laterally situated fields (cortex of the suprasylvian gyrus and auditory cortex) gives as a result a larger area of claustral degeneration. The farther laterally and caudally the area of ablation, the farther downwards changes in the claustrum are found to occur.

On the basis of these observations it can be assumed that in the caudal part of the claustrum the cells connected with the visual cortex are found most dorsally, more ventrally — those with the cortex of the suprasylvian gyrus and still lower — those connected with the auditory cortex. Possibly the cells located lowest caudally in the claustrum which were not degenerated in our experiments may possess connections with the undamaged ventral part of the auditory cortex and with the insular cortex.

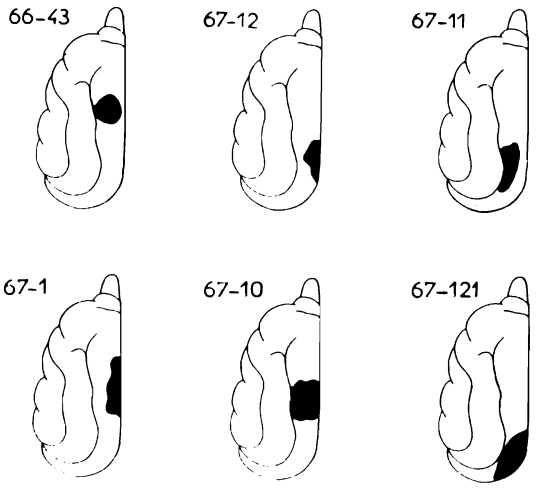

Fig. 6. The extent of cortical removals in cats 66-43, 67-12, 67-11, 67-1, 67-10 and 67-121. Other denotations as in Fig. 2.

The six cats with only a small area of the visual cortex removed form a separate group (Fig. 6). Three had part of visual cortex I removed (67–12, 61–1 and 67–121), in the others mainly visual cortex II was damaged (66–43, 67–11, 67–10). Despite careful examination of the whole claustrum and especially those areas which degenerate after abla-

tions of the whole visual cortex, no distinct degenerative changes were found to occur in the neurons, only here and there a slight gliosis or poorer stainability of single cells which also occurred in control cases and thus cannot be interpreted as being signs of degeneration. How can the lack of conspicuous cell degeneration following ablation of small areas of the visual cortex be explained? There are several interpretations. It can be assumed that cells of the dorsocaudal portion of the claustrum project into rather large areas of the visual cortex in a somewhat disseminated way. In order to produce degeneration in the claustrum it is not enough to injure a part of the visual cortex, but is necessary to remove it almost entirely. There is most probably a certain critical size of the area removed, below which degenerative changes in the claustrum are undiscernible because of their slightness. Irrespective of the interpretation it must be stated that after ablation of small areas of visual cortex in the claustrum, the location of the cells connected with specific parts of the visual cortex could not be found.

Intensity of degenerative changes in the claustrum. To trace the intensity of the degenerative changes in claustral cells relative to various periods of time, a group of animals with shorter survival periods was

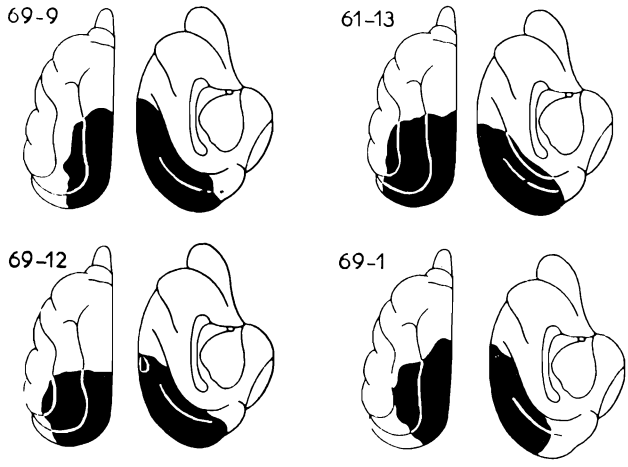

Fig. 7. The extent of cortical removals in cats 69-9, 69-12, 61-13 and 69-1. Other denotations as in Fig. 2.

studied. This group included four cats (Fig. 7): 69-9 (survival time 5 days), 69-12 (survival time 10 days), 69-13 (survival time 15 days) and 69-1 (survival time 24 days).

Cat 69–9 (survival time 5 days; Fig. 20). In small and medium enlargement no distinct signs of degeneration were apparent, only a slight

gliosis. The nerve cells appear somewhat rounded perhaps due to inadequately stained initial portions of dendrites. When greatly enlarged the distribution of Nissl granules seems to be slightly less regular.

Cat 69-12 (survival time 10 days; Fig. 21). Degenerative sings are visible and nerve cells smaller than on the unoperated side. In many places still unchanged neurons can be seen which form groups surrounded by glia cells which seem to be more numerous.

Cat 69-13 (survival time 15 days; Fig. 22). Degenerative changes similar to those observed in the previous case. A distinct gliosis can be seen, the nerve cells have decreased in size and a worse stainability of the cytoplasm is observed.

Cat 69–1 (survival time 24 days; Fig. 23). Degenerative changes are still more severe. With the exception of some cells which are slightly changed all the remaining neurons are badly degenerated, some consisting only of a weakly stained nucleus and a small rim of cytoplasm. Others have a little more cytoplasm but stain weakly and are barely visible in the Nissl picture. Some cells with a small amount of cytoplasm and very marked hyperchromatosis can be found. A high degree of gliosis can be found to occur.

In animals with a longer survival period changes are even more severe. In cat 67-99 (survival period 63 days; Fig. 24) gliosis is the leading symptom. There are only a few normal neurons within almost the entire area which is taken up by scattered glia cells and degenerated neurons. In some cases it is even difficult to determine whether we deal with a degenerated neuron or with a pathologically changed glia cell. In addition, neurons in process of disintegration surrounded by groups of glia cells are found. The number of nerve cells has definitely decreased.

Comparing the above results it has to be stated that the degeneration of the claustral cells following ablation of the visual cortex appears very early and its first not very perceptible signs may be observed already on the 10th day after the operation, while 15 days after surgery the degenerative changes are severe, gradually becoming more so and accompanied by a marked decrease in the size and number of neurons.

In order to get a better idea of the dynamics of claustral cell degeneration the same experimental material was used to compare degenerations in the claustrum and in the lateral geniculate body. It was aimed to determine whether the intensity and speed of changes are similar in both cases, or whether there are essential differences in the two processes.

Degenerations in the lateral geniculate body at various intervals of time are shown in Fig. 25 and 26. On comparing these changes with those in the claustrum (Fig. 19–24), a great convergence can be seen. In the early stages the extent of the degeneration is slightly greater in

the lateral geniculate body but later these differences disappear. A comparison of the dynamics of the changes in both centers is important when considering the type of degeneration in the claustrum and the direction of the fibers connecting it with the visual cortex.

Degeneration of axons and synapses in the claustrum following ablation of the visual cortex

Degenerations of the terminal parts of axons and synapses can best be shown by means of silver methods originating from the Nauta method and by electron microscopic examination.

Degeneration in specimes impregnated with silver nitrate by means of the Nauta and Fink–Heimer methods. Experiments were carried out by means of the silver nitrate method on six cats whose cortex was

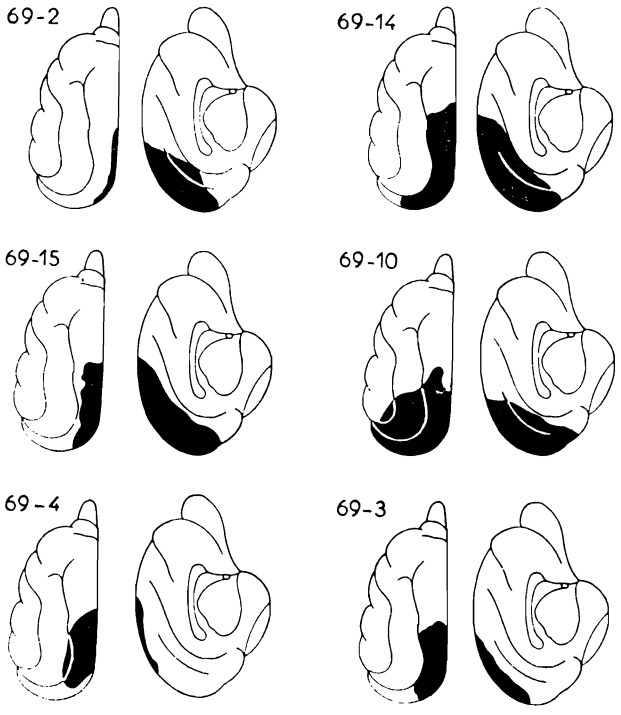

Fig. 8. The extent of cortical removals in cats 69-2, 69-15, 69-4, 69-14, 69-10 and 69-3. Other denotations as in Fig. 2.

removed to the extent shown in Fig. 8, their time of survival being 5–6 days. In all animals the cross-sections passing through the claustrum were impregnated with silver nitrate according to the Nauta method (Nauta 1957) or Fink-Heimer II modification (Fink and Heimer 1967).

In specimens impregnated by means of the Nauta method (Fig. 27) characteristic degenerations are found to occur in the dorsal part of the caudal portion of the claustrum, having the form of partly isolated fragments of axons which in many places look like beads. Here and there the ends of the degenerating axons are widened and resemble synaptic boutons. The degenerated axons penetrate into the claustrum from above and from the side of the external capsule, then generally run downwards or laterally, branching off in various directions and finally reach the claustral cells.

The degenerating axons revealed by the Nauta method are scattered somewhat irregularly. This would seem to suggest that the above mentioned fibers are not fibers of passage but end in the cells of the claustrum (terminal or preterminal degeneration). The degeneration of these axons made visible by means of the Nauta method is located in the same parts of the claustrum as that of the neural cells found in specimens stained by means of the Nissl method.

In specimens impregnated according to the Fink–Heimer II method (Fig. 25) the degeneration is also very characteristic. Sometimes structures similar to those found by means of the Nauta method can be seen. More often, however, there are some oval or round dark forms not connected with one another. Their size varies from 1 to 3 μm. They are scattered over the whole area of degeneration, the extent of which is similar to that observed in specimens stained according to the Nissl and Nauta methods.

Degeneration of claustral synapses in electron microscopic examination. In sections taken from animals with a survival period of 3–5 days degenerations of the preterminal portions of axons and synaptic boutons were seen to occur (Fig. 29). In the degenerating axons the axoplasm was generally dark. The degenerating synaptic boutons were transformed into dark structures of irregular shape. In some boutons synaptic vesicles and mitochondria were visible, in others the whole bouton formed a uniform dark mass indistinguishable from the degenerated axon. The whole picture presented severe degenerative changes.

DISCUSSION

The localization of the degeneration. The site of the degeneration found in the claustrum following the removal of the visual cortex agrees with the general topography of connections between the claustrum and the neocortex (Narkiewicz 1964, Carman et al. 1964). According to this scheme the anterior parts of the claustrum are connected with the cortical fields situated most rostrally and the caudal parts with the posterior

cortical areas. A similar dependence of the claustral cells on the cortical fields is found in the dorsoventral direction; the upper part of the claustrum is connected with the fields situated dorsally or more medially (near the interhemispheric fissure), while the lower part — with the cortical fields situated lower, near the insular cortex. Since the visual cortex occupies the dorsocaudal areas of the hemisphere of the brain, the degeneration in the dorsocaudal zone of the claustrum conforms to this scheme.

Degeneration of the cells. On basis of hitherto conducted investigations degenerations of claustral cells were found only in periods exceeding 4 weeks after removal of the cortex. Our material showed evidence of degeneration much earlier than that, already during the first weeks following surgery, which proves the rapidity of the process.

Discovering these changes at an early stage will lead to a better understanding of their nature. The question is whether we deal with a retrograde or a transsynaptic degeneration. The answer is of the utmost significance for the determination of the direction of the axons connecting the visual cortex with the claustrum. Finding that the above changes are degenerations would point to the existence of claustrocortical axons (fasciculi claustrocorticales) emerging from the claustral neurons (Fig. 9*A*). On the other hand if these changes are transsynaptic

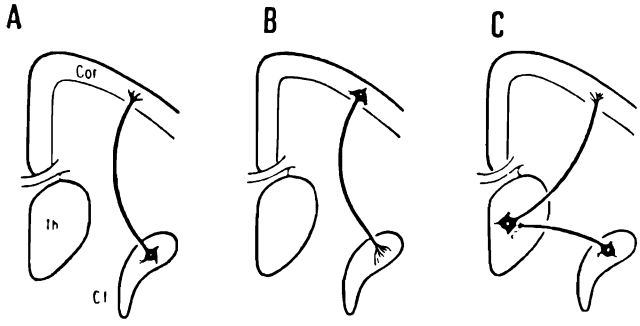

Fig. 9. The possible connections of the cortex with the claustrum: *A*, claustrocortical axons; *B*, corticoclaustral axons; *C*, indirect connections of the claustrum with the cortex through the thalamic nuclei.

ones this would suggest the existence of corticoclaustral connections (fasciculi corticoclaustricales) (Fig. 9*B*). The transsynaptic degeneration would have the following course: ablation of visual cortex with cells emitting axons to the claustrum would cause secondary (Wallerian) degeneration of the corticoclaustral axons followed by transsynaptic degeneration of claustral cells through synapses of these axons.

The differentiation of retrograde degenerations from transsynaptic

ones is not always easy as the course of both processes depends on many factors, among others on the age and species of the animal studied. Retrograde degeneration progresses much faster in very young animals, in which the neurons may disintegrate already within a few days following surgery (Brodal 1939). Similarly transsynaptic degeneration progresses much faster and is much more apparent in kittens than in adult cats (Wiesel and Hubel 1963).

The period of time it takes for the cellular degenerative changes to appear in various species is not uniform. Retrograde degeneration is manifest in the lateral geniculate body of the rabbit earlier than in the cat (Chow and Dewson 1966). Transsynaptic degeneration in the same center progresses very slowly in the rat (Tsang 1937) and rabbit (Cook et al. 1951), slightly faster in the cat (Cook et al. 1951), while in the macaque monkey it appears already during the first weeks (Mathews 1964, Beresford 1966).

Taking these differences into consideration our results can be compared only with those which concern degenerations in the nerve centers of adult cats. Transsynaptic degeneration in this animal was studied most carefully in the visual and auditory systems. In the former, after removal of the eyeball discernable lesions in the cells of the lateral geniculate body are manifest in the adult cat only on the 30th day after surgery (Cook et al. 1951), while during several months there is no evident decrease in the number of cells but merely a decrease in size. In the auditory system, after cutting the vestibulocochlear nerve also a transsynaptic degeneration occurs, i.e. a decrease in size of the cells which two months later are about 13–35% of their original size (Powell and Erulkar 1962). No loss of neurons was observed. Similar transsynaptic changes are found in other areas. But it must be stressed that so far transsynaptic degenerations in adult individuals were not found to lead to severe changes with degeneration of neurons in so short a period of time as that observed by us in the claustrum.

The retrograde degeneration may cause drastic changes to occur. This concerns among others the thalamus with the lateral geniculate body in which the neurons may be destroyed during just a few days after the removal of the cortex (Chow and Dewson 1966).

The present observations show that after the removal of the visual cortex together with the neighbouring areas the degeneration in the claustrum has a similar course to that in the lateral geniculate body. Although the changes in the claustral cells are less marked during the first days, later they become rapidly exacerbated and in the second month a conspicuous loss of neurons can be observed. A generally similar course of degeneration in the claustrum and in the lateral geni-

culate body, following the removal of the visual cortex, the severity of cellular changes, their early occurrence and a definite decrease in the number of neurons favor the hypothesis that the degeneration of the claustral cells is of the retrograde type.

This proves, as it has already been mentioned, that claustrocortical connections do exist. However, this does not exclude the possibility of the coexistence of corticoclaustral connections, about which more shall be said later. Also it should be mentioned that the degeneration of the claustral cells may be of the retrograde transsynaptic type (Narkiewicz 1964, 1966). By this it should be understood that after the removal of the visual cortex first the cells of the lateral geniculate body undergo retrograde degeneration and then the cells of the claustrum. This type of retrograde degeneration transgressing the synapses in the lateral geniculate body would point to the existence of axons running from the claustrum to the lateral geniculate body. Naturally, then there might be no direct connections between the claustrum and the visual cortex at all, only indirect ones, by way of the lateral geniculate body (Fig. 9*C*). But only slight difference in time between the degeneration in the claustrum and in the lateral geniculate body and the fact that it has been found that the destruction of the latter does not cause changes in the claustral cells definitely disproves this (our unpublished data).

Degeneration of axons and synapses. The degeneration of the axons found by the authors in the claustrum after removal of the visual cortex agrees, in principle, with the changes described by Druga (1968). He found in cats on the 6–8th day following surgery in sections prepared by means of the Nauta method a characteristic pattern in the form of elongated dark segments which are in accordance with degenerating terminal portions of axons. The results of the present work supplement these previous findings as the changes which have been found by means of the electromicroscope examination have not been described before.

The results obtained by means of the Nauta method as well as those obtained by means of electromicroscope examinations are consistent. The removal of the visual cortex is followed by degeneration of axons and synaptic boutons. In principle, the fact of the occurrence of a degeneration of this type ought to pint to the existence of axons emerging from cortical cells and ending synaptically on the cells of the claustrum. But in the case of centers in which severe degenerations of cells appear soon and this is so far the claustrum, the matter is more complicated, as there is a possibility that the degenerated synaptic boutons are not the ends of corticoclaustral axons, but collaterals of the claustral cells disintegrating as a result of retrograde degeneration. In that case the course

of the degeneration would be different: at first the degeneration of claustral cells as a result of the destruction of the terminal portions of their axons, then degeneration of the collaterals together with synaptic boutons.

But the early appearance of marked axon degeneration in the claustrum visible on the 5th day, while changes in the claustral cells are not yet evident, seems to favor the hypothesis that the degenerating axons are corticoclaustral rather than claustrocortical.

On basis of degeneration of cells as well as that of axons it is very probable that the visual cortex and the claustrum are reciprocally interconnected.

SUMMARY

1. After removal of the visual cortex a marked degeneration of the nerve cells in the dorsocaudal part of the claustrum is observed.

2. Degeneration of the claustral cells appears as early as 10 days after surgery. Later the degenerative changes gradually become exacerbated, the perikarya decrease in size and number and two months later only single cells are found unchanged in the degenerative areas.

3. The rapidity of these changes as well as their similarity to the course of degeneration in the lateral geniculate body favors the hypothesis that the degeneration of claustral cells is retrograde, which would seem to prove the existence of axons running from the claustrum to the visual cortex (fasciculi claustrocorticales).

4. By means of the Nauta and Fink-Heimer methods 5–6 days after removal of the visual cortex changes characteristic for degeneration of terminal parts of axons can be found. These changes appear in the same area of the claustrum as the degeneration of neurons.

5. Electron microscopic examination 5 days after surgery revealed degeneration of synaptic boutons in the dorsocaudal part of the claustrum.

6. Degeneration of synapses and preterminal parts of axons in the claustrum resulting from the ablation of the visual cortex favors the existence of corticoclaustral axons (fasciculi corticoclaustrales).

7. Both the claustrocortical and corticoclaustral axons connect the visual cortex with the same dorsocaudal portion of the claustrum. This proves the close relationship of this part of the claustrum to the visual system.

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REFERENCES

BERESFORD, W. A. 1966. An evaluation of neuroanatomical methods and their relation to neurophysiology. Brain Res. 1: 221–249.

BERLUCCHI, C. 1927. Richerche di fine anatomia sul claustrum e sull' insula dell gatto. Riv. Sper. Freniat. Med. Leg. Alien. Ment. 51: 125–157.

BROCKHAUS, H. 1940. Die Cyto- und Myeloarchitektonik des Cortex claustralis und des Claustrum beim Menschen. J. Psychol. Neurol. 49: 249–348.

BRODAL, A. 1939. Experimentelle Untersuchungen über retrograde Zellveränderungen in der unteren Olive nach Läsionen des Kleinhirns. Z. Ges. Neurol. Psychiat. 166: 646–704.

CARMAN, J. B., COWAN, W. M. and POWELL, T. P. S. 1964. The cortical projection upon the claustrum. J. Neurol. Neurosurg. Psychiat. 27: 46–51.

CHOW, K. L. and DEWSON, I. H. 1966. Numerical estimates of neurons and glia in lateral geniculate body during retrograde degeneration. J. Comp. Neurol. 128: 63–74.

COOK, W. H., WALKER, J. H. and BARR, M. L. 1951. A cytological study of transneuronal atrophy in the cat and rabbit. J. Comp. Neurol. 94: 267–292.

DRUGA, R. 1966*a*. Cortico-claustral connections. I. Fronto-claustral connections. Folia Morphol. (Praha) 14: 391–399.

DRUGA, R. 1966*b*. The claustrum of the cat. Folia Morphol. (Praha) 14: 7–16.

FINK, R. P. and HEIMER, L. 1967. Two methods for selective silver impregnation of degenerating axons and their synaptic endings in the central nervous system. Brain Res. 4: 369–374.

GURDJIAN, E. S. 1928. The corpus striatum of the rat. J. Comp. Neurol. 45: 249–281.

HUMPHREY, T. 1936. The telencephalon of the bat. I. The non-cortical nuclear masses and certain pertinent fiber connections. J. Comp. Neurol. 65: 603–711.

LANDAU, E. 1936. Quelques nouvelles considerations sur l'avant mur. Arch. Anat. (Strasbourg) 23: 165–181.

LANDAU, E. 1938. Le claustrum parvum chez l'Homme. Mem. Soc. Vaud. Sci. Nat. 41: 45–62.

MATHEWS, M. R. 1964. Further observations on transneuronal degeneration in the lateral geniculate nucleus of the macaque monkey. J. Anat. 98: 255–263.

NARKIEWICZ, O. 1964. Degenerations in the claustrum after regional neucortical ablations in the cat. J. Comp. Neurol. 123: 335–356.

NARKIEWICZ, O. 1966. Connection of the claustrum with the cerebral cortex (in Polish) Folia Morphol. (Warsaw) 25: 554–561.

NAUTA, W. J. H. 1957. Silver impregnation of degenerating axons. *In* W. F. Windle (ed.), New research techniques of neuroanatomy. Charles C Thomas, Springfield, p. 17–26.

PILLERI, G. 1961. Zur Struktur des Claustrum bei *Didelphis marsupialis* Lin. Acta Anat. 45: 310–314.

PILLERI, G. 1962. Das Claustrum des Kanadischen Bibers. Struktur und vergleichende Anatomie. J. Hirnforsch. 5: 59–81.

PINTUS, G. S. 1930. Struttura cellular e citoarchitettura dell' antimuro umano. Riv. Neurol. 3: 289–312.

PINTUS, G. S. 1931. Forma e connessioni grigie dell' antimuro umano. Arch. Gen. Neurol. Psichiat. 12: 16–28.

PINTUS, G. S. 1932. Connessioni dell' antimuro umano. Arch. Gen. Neurol. Psichiat. 13: 9–20.

POWELL, T. P. S. and ERULKAR, S. D. 1962. Transneuronal cell degeneration in the auditory relay nuclei of the cat. J. Anat. 96: 249–268.

RAE, A. S. L. 1954*a*. The form and structure of the human claustrum. J. Comp. Neurol. 100: 15–40.

RAE, A. S. L. 1954*b*. The connections of the claustrum. Confinia Neurol. 14: 211–219.

ROSE, M. 1928. Die Ontogenie der Inselrinde. Zugleich ein Beitrag zur histogenetischen Rindeneinteilung. J. Psychol. Neurol. 36: 182–209.

STELMASIAK, M. 1955. The volume of the claustrum in the human cerebral hemispheres (in Polish). Folia Morphol. (Warsaw) 6: 137–144.

TENEROWICZ, M. 1960. The morphology and topography of the claustrum in the brain of the dog. Acta Biol. Cracov. 3: 105–113.

TSANG, Y. C. 1937. Visual centres in blinded rats. J. Comp. Neurol. 66: 211–261.

VRIES, E. de 1910. Bemerkungen zur Ontogenie und Phylogenie des Claustrums. Folia Neurobiol. 4: 481–513.

WIESEL, T. N. and HUBEL, D. H. 1963. Effects of visual deprivation on morphology of cells in the cat's lateral geniculate body. J. Neurophysiol. 26: 978–993.

YOUNG, M. W. 1936. The nuclear pattern and fiber connections of the noncortical centers of the telencephalon of the rabbit. J. Comp. Neurol. 65: 295–401.

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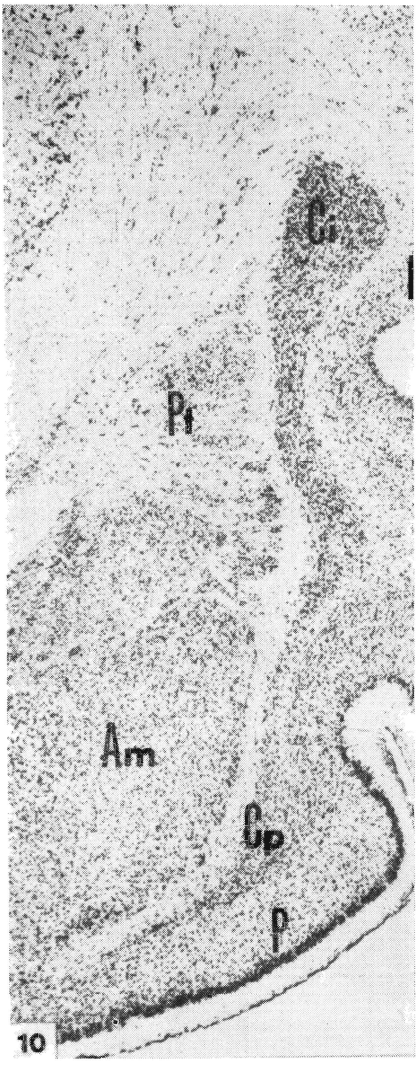
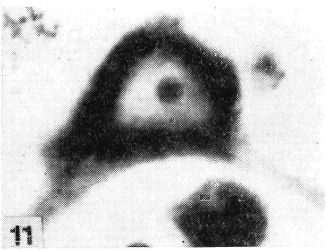
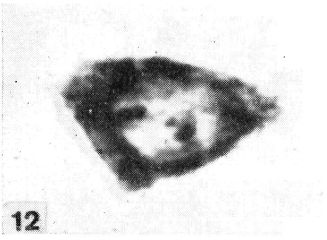
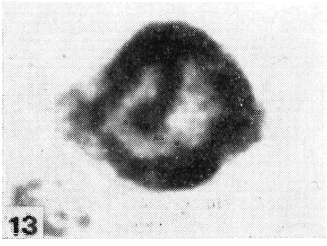








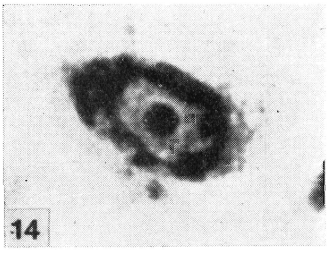


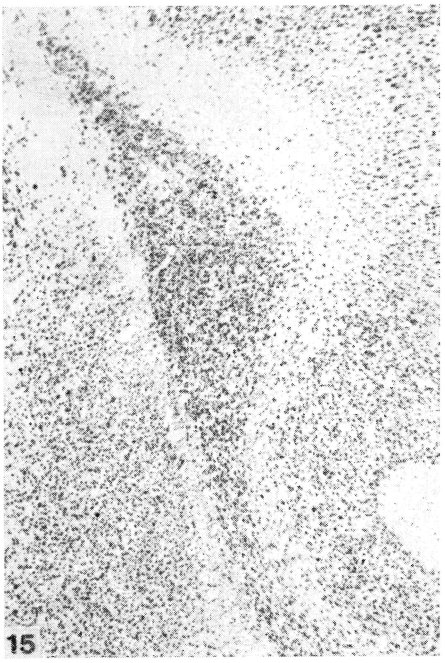
Fig. 10. Photogram of a cross-section through the caudal portion of the claustrum: *Am*, corpus amygdaloideum; *Ci*, claustrum insulare; *Cp*, claustrum prepiriforme; *I*, insula; *P*, cortex prepiriformis; *Pt*, putamen. Cresyl violet. × 11.

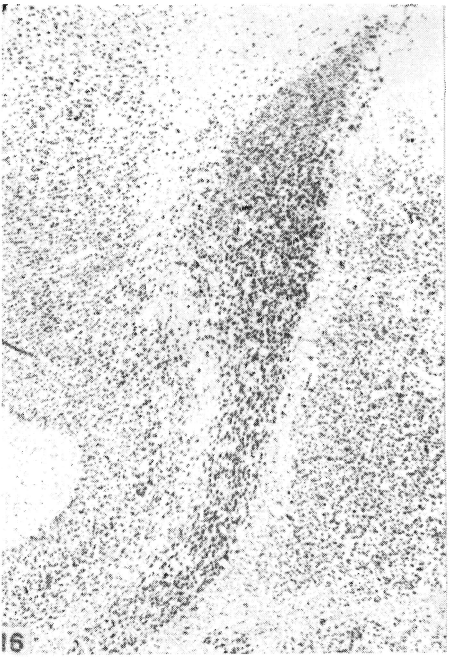
Fig. 11. The multipolar cell of the cat's claustrum. Cresyl violet. × 900.

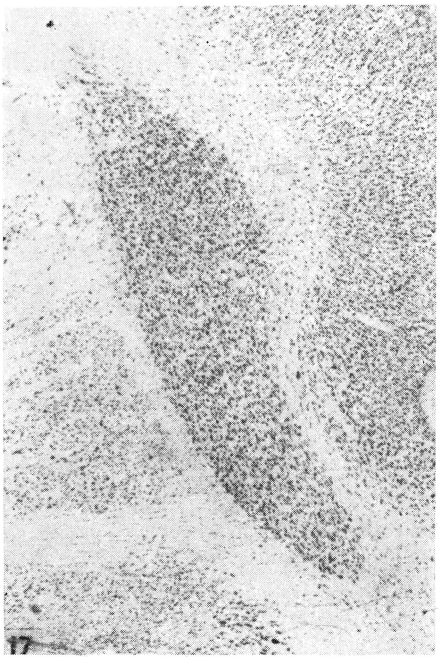
Fig. 12. The pyramidal cell of the cat's claustrum. × 900.

Fig. 13. The round cell of the cat's claustrum. × 900.

Fig. 14. The fusiform cell of the cat's claustrum. × 900.







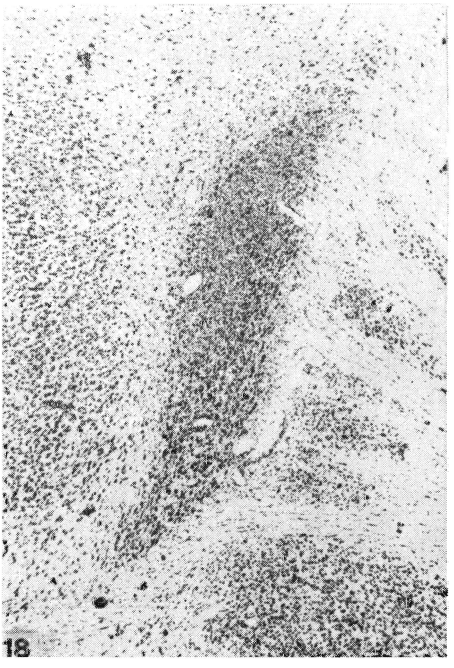


Fig. 15–18. Cross-sections through the caudal portion of the claustrum in cat 67-99.
Fig. 15 and 17. Normal (unoperated) side.
Fig. 16 and 18. Operated side. Note severe degeneration of the neurons combined with gliosis.

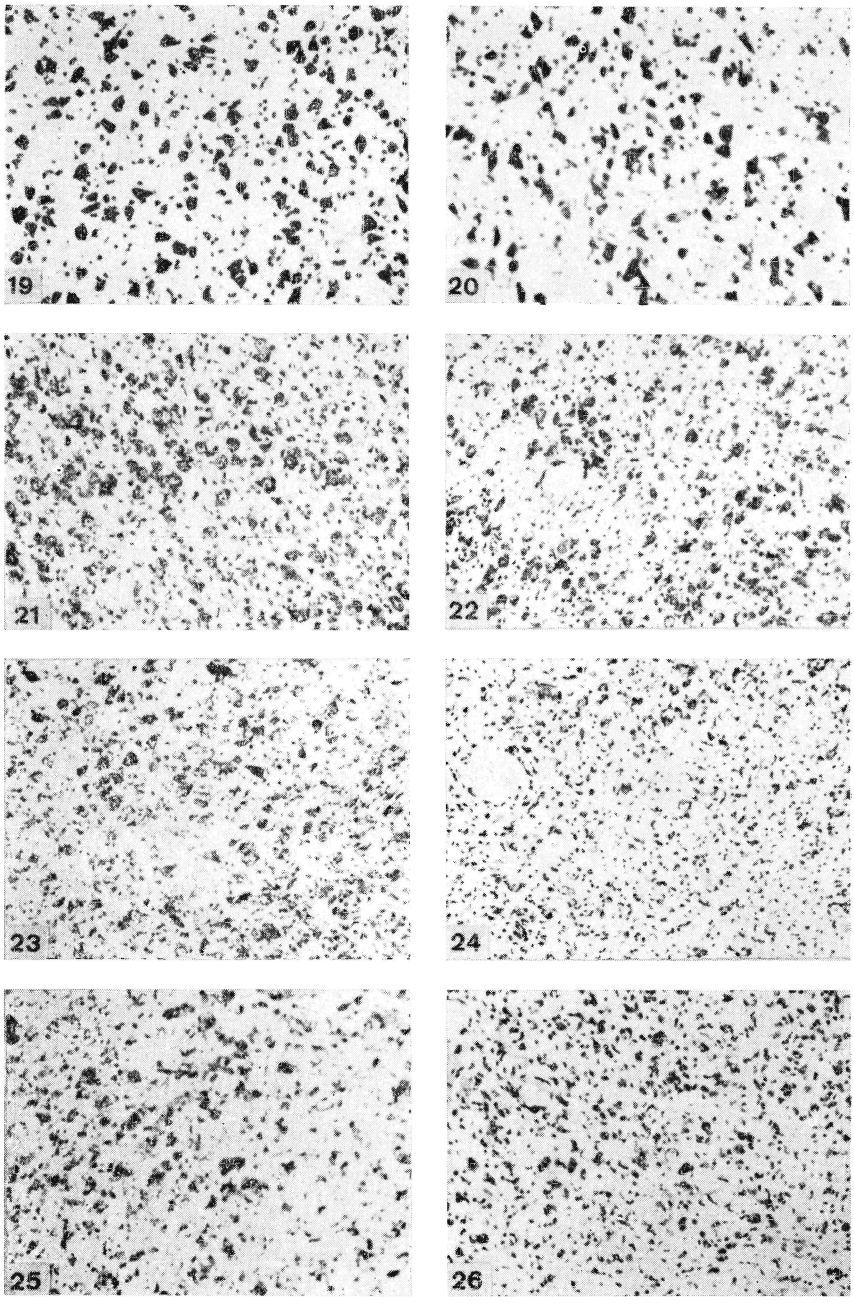
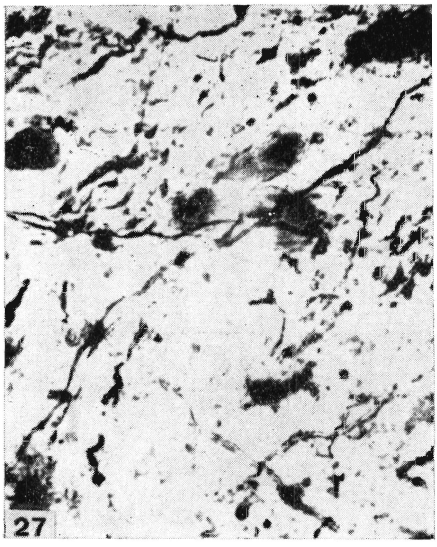
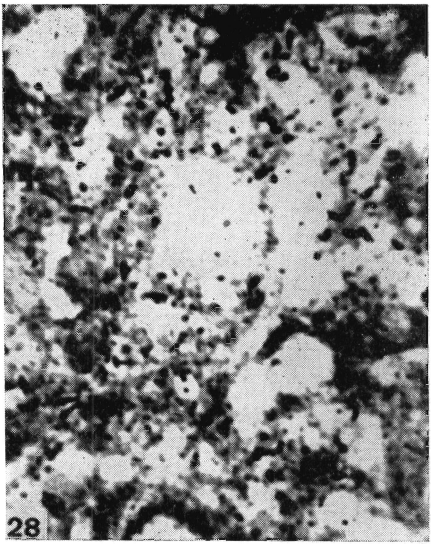


Fig. 19–24. Microphotograms of the dorsocaudal portion of the claustrum.
Fig. 19. Normal (unoperated) cat. Fig. 20. Cat. 69-9 (5 days survival). Fig. 21. Cat 69-12 (10 days survival). Fig. 22. Cat 69-13 (15 days survival). Fig. 23. Cat 69-1 (24 days survival). Fig. 24. Cat 67-99 (63 days survival).
Fig. 25 and 26. Microphotograms of the borderline between lamina A and A_1 of the lateral geniculate body.
Fig. 25. Cat 69-12 (10 days survival). Fig. 26. 69-1 (24 days survival). Cresyl violet. $\times$ 400.





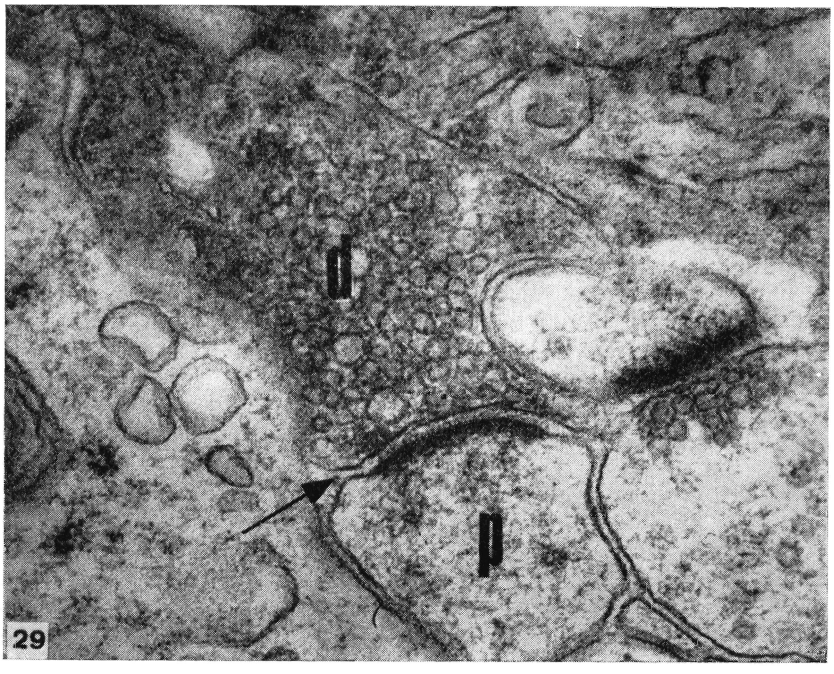


Fig. 27. Cat 69-14. Dorsocaudal portion of the claustrum. Nauta method; × 900. Note the black fragments of degenerating axons. Few normal fibres. Some neurons and glia cells visible as larger dark irregular spots.

Fig. 28. Cat. 69-14. Dorsocaudal portion of the claustrum. Fink-Heimer II method. × 900. Small, black, round or oval structures corespond to degenerating preterminal portions of the axons or terminal boutons.

Fig. 29. Electronogram of the dorsocaudal portion of the claustrum following visual cortex removal. Five days survival time. × 49000. Note the dark degenerating terminal bouton (*d*) with aggregated vesicles of various size. The synaptic cleft (arrow) and postsynaptic portion (*p*) unchanged.