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## **Demonstration of Bilateral Claustro-Cortical Connections in the Cat with the Method of Retrograde Axonal Transport of Horseradish Peroxidase**

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**Summary.** The claustrum of the cat was studied to determine the extent of its projection to the cerebral cortex. Neurons in the bilateral dorsal claustrum are labeled by retrograde axonal transport of horseradish peroxidase (HRP) from injection sites of different neocortical areas with preponderance to the ipsilateral side. It is revealed that the dorsal claustrum projects to almost entire regions of the bilateral neocortex and that distribution of labeled neurons in the bilateral claustrum has topographical correspondence, rostro-caudal as well as dorso-ventral, with regard to the sites of injections in the neocortex. No neurons in the ventral claustrum are labeled.

The claustrum is a sheet of gray matter located beneath the lateral portion of the cerebral cortex. Previous authors (CARMAN, COWAN and POWELL, 1964; DRUGA, 1966, 1968, 1971; NORITA and FUKUSHIMA, 1976) have reported that the claustrum receives fibers from extensive areas of the ipsilateral cerebral cortex with a well-defined topographic pattern. Recently, KÜNZLE (1975) found the bilateral cortico-claustral projections in the monkey by using autoradiographic technique.

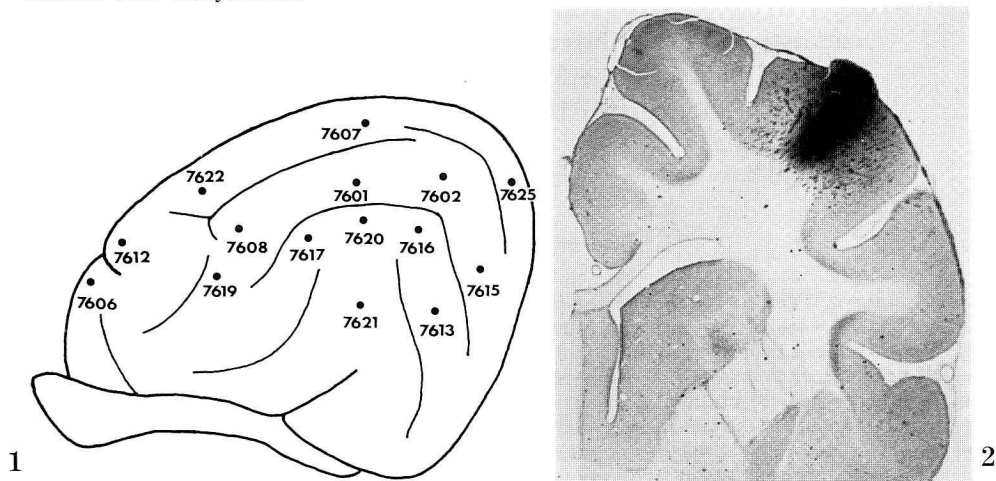
On the other hand, only a few morphological data are available concerning the claustrum-cortical connections. On the basis of the retrograde cellular degeneration method, NARKIEWICZ (1964) demonstrated claustral projections to the cat neocortex. He showed only ipsilateral projections in this system and concluded that the claustrum-cortical projections are topographically organized.

In an attempt to get more detailed information of the claustrum-cortical projection, the present study is carried out with the method of retrograde axonal transport of HRP as a tracer.

### **Material and Methods**

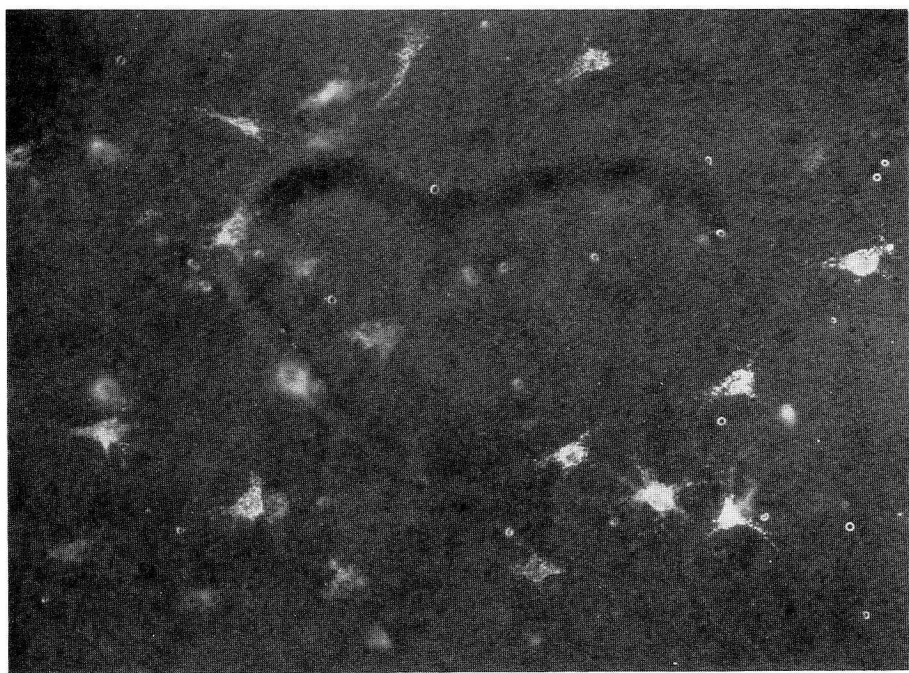
Under pentobarbital sodium anesthesia, 0.25–0.4  $\mu$ l of 30% HRP (Type VI, Sigma) was injected, by using a 1  $\mu$ l Hamilton syringe (7001–N), into different cortical areas of 15 adult cats. In all cases, 24 hrs after the injection, the cats were anesthetized with pentobarbital sodium and perfused through the left cardiac ventricle with Ringer's solution followed by a fixative containing 0.4% formaldehyde and 1.25% glutaraldehyde in 0.1 M phosphate buffer. The brains were dissected out, kept in the fixative (4°C) for 12 to 24 hrs and stored overnight in the refrigerator in 0.1 M phosphate buffer containing 5% sucrose. Sections (60–80  $\mu$ m) were cut coronally on the freezing microtome and they were transferred to a solution containing 0.05% 3, 3'-diaminobenzidine tetrahydrochloride in Tris HCl buffer (pH 7.6) for 30 min at room temperature. Further, they were incubated for 30 min at room temperature in a second solution containing the same concentration of substrate in Tris HCl with

0.03 ml of 30% hydrogen peroxide per 100 ml. Some of them were lightly counter-stained with cresylviolet.



**Fig. 1.** Diagram showing the sites of HRP injection on the lateral surface of the cerebrum performed in this study (15 cases). Figures indicate the animal numbers.

**Fig. 2.** A photomicrograph showing the maximal diffusion of HRP following an injection ( $0.25 \mu\text{l}$ , survival 24 hrs) in Cat 7608. Cresylviolet counterstain.  $\times 3.1$



**Fig. 3.** A low magnification photomicrograph showing HRP-positive neurons in the claustrum. Dark-field illumination.  $\times 440$

Nomenclature of the cat gyri used in this paper are based upon the map of KAWAMURA (1971, 1973).

### Results

Injection sites of HRP were restricted to the small regions in the cerebral neocortex (Fig. 1). A photograph of a representative injection site is shown in Figure 2. In most cases, diffusion of the HRP in the injection site was almost confined to the gray matter, but in some cases it slightly involved the underlying white matter (Fig. 5A-F).

In the dorsal claustrum ipsilateral to the injection site, many neurons contained a yellow-brownish granular reaction product in the perikarya as well as in the proximal dendrites (Fig. 3). These labeled neurons were found to be of various types (Fig. 4a-c) with exception of the small size, described by NORITA and HIRATA (1976). Although smaller in number, the labeled neurons were found in contralateral claustrum, showing a similar distribution pattern to that found in the ipsilateral side. The ventral claustrum contained no labeled cells.

For convenience sake, description will be given below dividing the cortical areas into four groups as the somatic sensorimotor, the visual, the auditory and the "association" cortex.

#### Somatic sensorimotor cortex

HRP-injections were made in the anterior sigmoid (Cat 7606), the posterior sigmoid (Cat 7612) and coronal (Cat 7619) gyri in three cases (Fig. 1).

In Cat 7606, the cortical site with a high concentration of the injected HRP (indicated by black) as well as the area of its diffusion (dotted) is indicated in Figure 5A. In this case, the labeled neurons in the claustrum are chiefly located in its rostral third portion (Fig. 6).

Following an injection of HRP into the coronal gyrus (Cat 7619, Fig. 1), the labeled cells are also found mainly in the rostral third of the claustrum but more caudal than in Cat 7606 (Fig. 6).

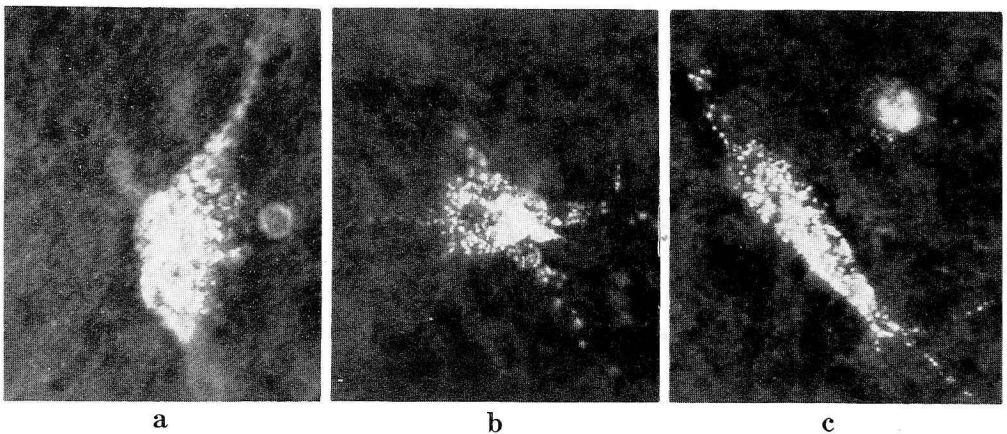


Fig. 4. Photomicrographs of large (a), medium (b) and fusiform (c) HRP-positive neurons in the claustrum. Dark-field illumination.  $\times 1,500$

Findings obtained in Cat 7612 (not illustrated) are similar to those in Cat 7606.

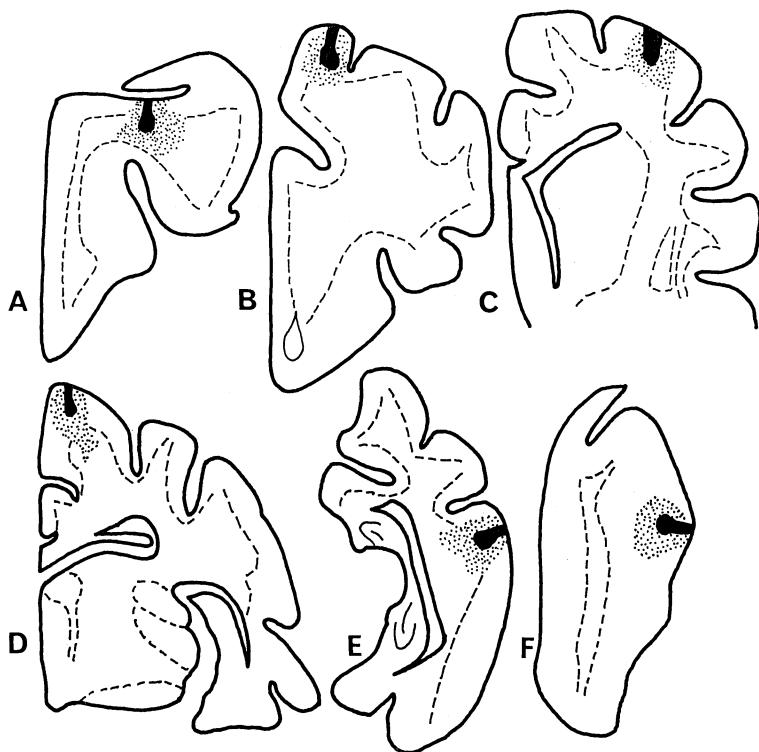
### Visual cortex

In Cat 7607 (Fig. 5D), an injection of HRP was made in the middle portion of the anterior lateral gyrus, corresponding to the visual area II of WOOLSEY (1947). Labeled cells are found in the middle third of the rostro-caudal extent of the claustrum, and they are confined to its dorsal margin (Fig. 6).

The injection site in Cat 7615 (Fig. 5F) is in the posterior suprasylvian gyrus (corresponds to the visual area II of WOOLSEY, 1947). Labeled neurons are found mainly in the caudal third of the dorsal claustrum, extending in areas located more ventrally than in the case of Cat 7607 (Fig. 6).

In Cat 7602 an injection was made in the caudal-most portion of the middle suprasylvian gyrus (Fig. 1). Most labeled neurons are found in the middle third of the rostro-caudal extent of the claustrum (not illustrated).

Following an injection of HRP into the posterior lateral gyrus (Cat 7625, Fig. 1), corresponding to the visual area I of WOOLSEY (1947), labeled neurons are mostly found in the caudal quarter of the claustrum (not illustrated).



**Fig. 5.** Diagram (coronal sections) showing the maximal extent of the injection of HRP in cases of Cat 7606 (A), 7622 (B), 7608 (C), 7607 (D), 7616 (E) and 7615 (F).

High concentration of HRP injection areas is black and its spreading is stippled.

### Auditory cortex

Injections of HRP were performed in the anterior (Cat 7617) and the middle (Cat 7620) portions of the middle ectosylvian gyrus (Fig. 1), corresponding to the A I area of WOOLSEY (1947). In these two experiments most of the labeled neurons in the claustrum are found in its middle third of the rostro-caudal extent (Fig. 7).

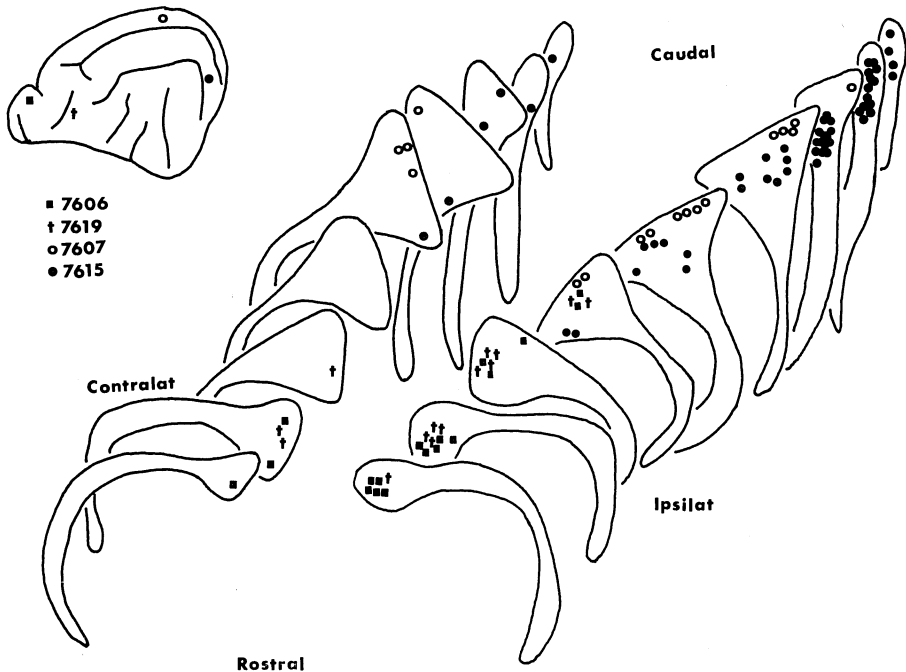
Distribution of the neurons labeled after an injection in the middle sylvian gyrus (Cat 7621, Fig. 1), is similar to the pattern resulting from that in Cat 7617 and 7620, but occupies a more ventral part in the dorsal claustrum.

Following an injection (Fig. 5E) into the most posterior portion of the middle ectosylvian gyrus (Cat 7616, corresponding to the Ep I of LILLY, 1951), numerous labeled neurons are found in the middle third of the rostro-caudal extent of the dorsal claustrum, and some other HRP-positive cells are likewise found in fairly extensive areas (Fig. 7).

An injection was made in the middle portion of the posterior ectosylvian gyrus (Cat 7613, Fig. 1, Ep II of LILLY, 1951). In this case distribution of labeled neurons in the claustrum is similar to that in Cat 7616.

### "Association" cortex

HRP-injections were made in the following area: the rostral portion of the anterior lateral (Cat 7622, Fig. 1, 5B, the anterior lateral association area of THOMPSON,



**Fig. 6.** Diagrammatic representation of the bilateral distribution of HRP-positive neurons within the claustrum following injections of HRP in the somatic sensorimotor (Cat 7606 ■, 7619 ▲) and the visual (Cat 7607 ○, 7615 ●) cortex. In this and subsequent figures, location of HRP-positive neurons in the claustrum is indicated by using different symbols.

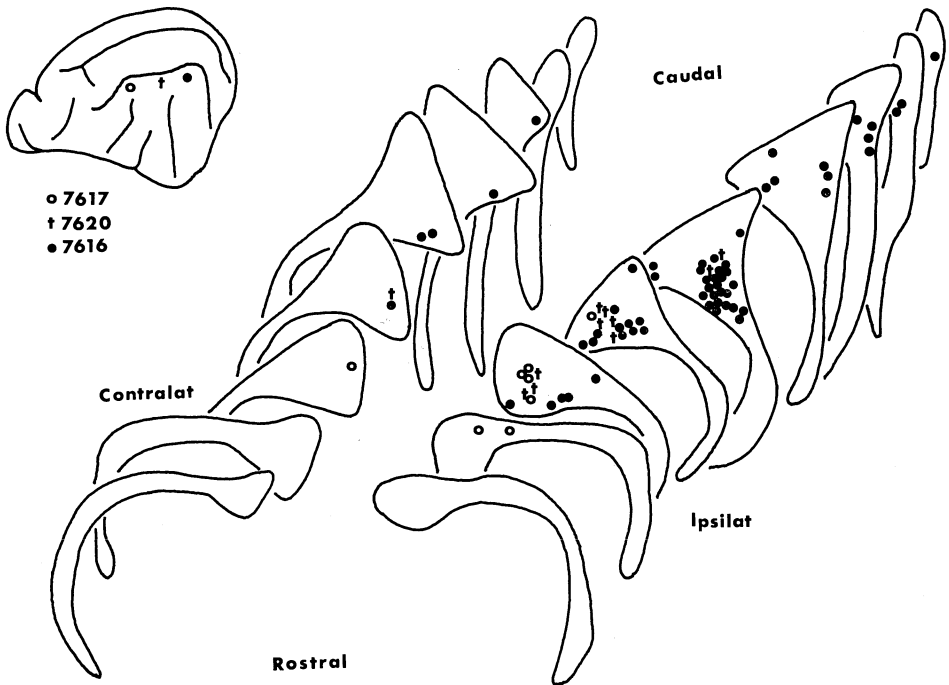


Fig. 7. Distribution of labeled neurons following injections of HRP in the auditory cortex.

JOHNSON and HOOPES, 1963), the anterior suprasylvian (Cat 7608, Fig. 1, 5C, the area 5 of HASSLER and MUHS-CLEMENT, 1964) and the middle portion of the middle suprasylvian (Cat 7601, Fig. 1, the anterior part of area 7 of GUREWITSCH and CHATSCHATURIAN, 1928) gyrus.

In these three cases, labeled neurons are found mostly in the middle third of the dorsal claustrum (Fig. 8).

Findings obtained from the present study indicate that neurons in the bilateral dorsal claustrum give off their axons to an extensive area of the cerebral neocortex and that some degree of topographic arrangements, both rostro-caudal and dorso-ventral, exist in this projection system.

### Discussion

Investigations with the anterograde degeneration methods (CARMAN, COWAN and POWELL, 1964; DRUGA, 1966, 1968, 1971; KEMP and POWELL, 1970) have shown the presence of cortico-claustral fibers which arise from almost the entire cortex. Electron microscopic examination of the cat claustrum after-lesions of the cerebral cortex have shown that degenerating axons end predominantly upon dendritic spines and less frequently upon dendritic shafts and cell somata (JURANIEC, NARKIEWICZ and WRZOLKOWA, 1971; NORITA and FUKUSHIMA, 1976). In addition, the claustrum has been known to receive afferents from various sources other than the cerebral cortex, e.g. the striatum and the substantia nigra (ANDERSEN, 1968), the nuclei centralis medius, submedius and reuniens (NAUTA and WHITLOCK, 1954) and locus caeruleus (PICKEL, SEGAL and

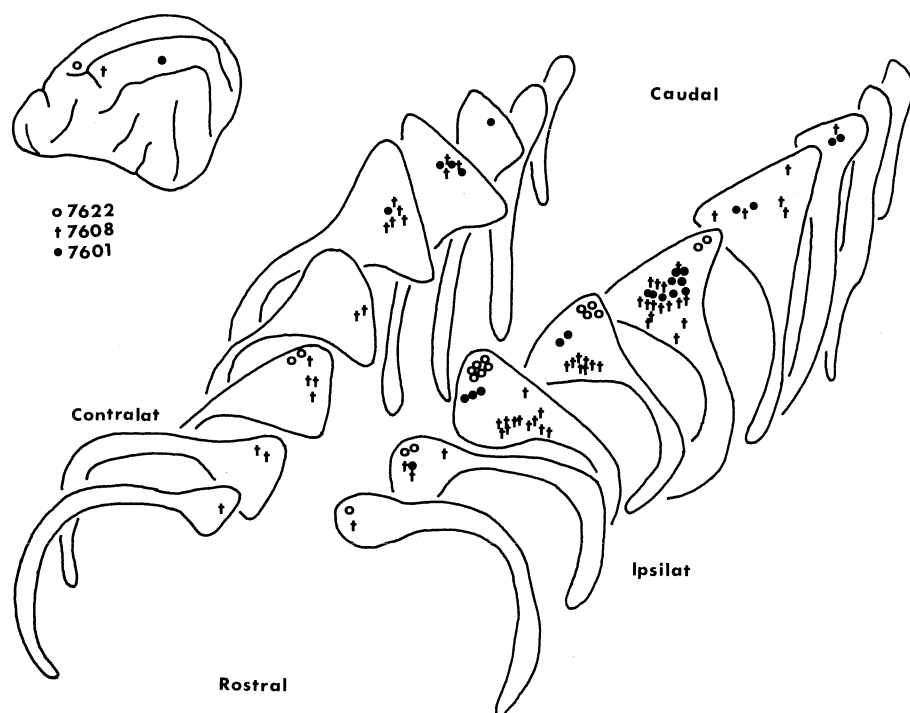


Fig. 8. Distribution of labeled neurons following injections of HRP in the "association" cortex.

BLOOM, 1974). Electrophysiologically, SPECTOR, HASSMANNOVA and ALBE-FESSARD (1970) showed that the somatic sensory, auditory and visual projections converge upon the claustrum via multiple and complex pathways, and that the dissimilar responses can be obtained in various portions of the claustrum when sites of stimulation differ. Thus, they concluded that the claustrum is functionally a non-homogeneous multisensory structure, and divided into three subdivisions as anterior, intermediate and posterior. Moreover, investigating the responses of individual claustral neurons to somatic, auditory and visual stimuli, the same authors (1974) stated that sensory afferents from various origins are distributed differently inside the structure and terminate in different combinations upon individual neurons. In our Golgi study in the claustrum (unpublished data), we found that there are many Golgi type II as well as Golgi type I neurons and that the latter send their numerous axon collaterals within the claustrum prior to going out to this gray matter, although little is known of the intrinsic connections of the claustral neurons.

The present study reveals that neurons in the dorsal claustrum are bilaterally labeled, following injections of HRP into various cortical areas, with preponderance to the ipsilateral side. In addition, in cases where injections are made in the somatic sensorimotor (Cat 7606, 7612, 7619), the first (Cat 7617, 7620) and the second (Cat 7621) auditory areas, the labeled cells in the claustrum are less in number than in cases of other cortical injections. JONES and LEAVITT (1974) stated that the number of labeled cells is primarily dependent upon the number and concentration of the axon terminals in the vicinity of the injection site. It is suggested, therefore, that the dorsal claus-

trum projects their axons more densely to the ipsilateral than to the contralateral cortex, and that the claustrum projects their axons densely to the visual, Ep and "association" areas and sparsely to the other parts of the cortex, although it remains unclear whether all of the afferents to a given region will give a positive result with the retrograde axonal transport method (NAUTA, PRITZ and LASEK, 1974).

On the other hand, NARKIEWICZ (1964) showed cellular degeneration in the ipsilateral insular (dorsal) claustrum following lesions of almost the entire neocortical areas, and he claimed that the claustrum-cortical connections are topographically organized, although he only mentioned ipsilateral connections. The present study demonstrates that the ipsilateral as well as the contralateral claustrum-cortical projections show a certain degree of topographic organization. Labeling of a large number of neurons in the rostral third of the claustrum results from injections into the rostral portion of the cortex (e.g. anterior sigmoid gyrus, Cat 7606, see Fig. 6), and that in the caudal third of the claustrum mostly from injections into the occipital region (e.g. posterior suprasylvian gyrus, Cat 7615, see Fig. 6). Thus a topographic correspondence in the claustrum exists bilaterally along the rostro-caudal axis. Furthermore, it has become evident that the dorso-ventral topographic relationship is present bilaterally, although there is some overlap of the labeling of claustral neurons following injections to different cortical areas.

From the present findings, together with previous anatomical and physiological data, it is, therefore, supposed that the claustrum receives convergent information channels from almost entire regions of the cerebral cortex and from other structures mentioned above and, in turn, it projects back, directly or indirectly, to the cortex after certain modification and/or integration within the claustrum, although the precise mechanism of the claustrum still remains to be clarified.

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### ペルオキシダーゼ法によるネコ前障から両側大脳皮質への投射

車 田 正 男

ネコ前障から大脳新皮質への投射についてしらべた。大脳新皮質の種々の領域に HRP を注射したのち 前障を観察したところ、両側の背側前障に HRP で標示されたノイロンが認められ、注射した側の前障に認められたノイロンの数は 反対側のそれより多かった。さらに HRP で標示されたノイロンの前障内での分布は、注射された皮質との間で 吻一尾 および 背一腹 方向の局在関係を示した。腹側前障においては、HRP で標示されたノイロンを見いだすことができなかった。

従って 背側前障は ほとんどすべての両側大脳新皮質へ、局在関係をもちながら投射していることがわかった。



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