Claustrum in the Hedgehog (Erinaceus europaeus) Brain: Cytoarchitecture and Connections With Cortical and Subcortical Structures

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ABSTRACT

The cytoarchitecture of the claustrum in the hedgehog (Erinaceus europaeus) brain, the morphology of its neurons, and the efferent connections with cortical and subcortical structures were studied with the Nissl and Klüver-Barrera, the Golgi, and the horseradish peroxidase methods. It was found that the claustrum is a well developed nucleus in the hedgehog telencephalon and, as in other mammals, is divided into dorsal and ventral parts. In Golgi-stained sections, spiny multipolar cells are the predominant neurons of both the dorsal and the ventral claustrum and are projection neurons. Aspiny multipolar neurons with fewer, often beaded, dendrites constitute a minority in both divisions and are interneurons.

Injections of wheat germ agglutinin-horseradish peroxidase (WGA-HRP) in the prefrontal, motor, somatosensory, auditory and visual areas, and HRP or WGA-HRP injections in the thalamus showed that:

1. the claustroneocortical projections originate in the dorsal claustrum and are distributed to the entire neocortex; these projections are mainly ipsilateral but some also originate contraterally; (2) the claustroneocortical projections show a rough topographic organization; there exists a substantial degree of overlap; and (3) the claustrothalamic projection, arising throughout the dorsal claustrum, is strictly ipsilateral. No evidence of a thalamoclaustral projection was found.

The present results suggest that, although the hedgehog has been referred to as a "paleocortical mammal" owing to the great development of its rhinencephalic structures in comparison with its small neocortex, the dorsal claustrum is well developed and is connected with all neocortical areas as well as with the thalamus, establishing it as a key structure in the hedgehog forebrain.

Key words: insectivores, telencephalon, Golgi impregnation, neuronal morphology, WGA-HRP tracing

The claustrum is a telencephalic structure present in all mammals examined, from the insectivorous bats to primates and man. Two parts can be distinguished in all mammals: the dorsal claustrum (insular claustrum), which underlies the insular cortex, and the ventral claustrum (piriform claustrum), which adjoins the prepiriform cortex. The size and form, particularly of the dorsal claustrum, varies greatly throughout the mammalian phylogenetic scale. In lower mammals it is a small, ventrally located nucleus; in carnivores it is a structure that rivals, or even exceeds the size of the putamen, and in primates it is a slab, bounded laterally by the extreme capsule, and medially by the external capsule (Miodonski, '75; Olson and Graybiel, '80; Zilles et al., '80; LeVay and Sherk, '81a; Macchi et al., '81; Paxinos and Watson, '82; see also Sherk, '86, for review).

For many years the claustrum has received little attention by investigators and still remains poorly understood, whether in terms of its ontogenetic origin, its fiber connections, or its function. However, interest in this structure has been kindled by reports describing extensive reciprocal connections with the neocortex (Jayaraman and Updyke, Accepted October 12, 1991.
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claustrum has been noted by others (Valverde and Facal-Valverde, '86) and in our previous papers (Papadopoulos et al., '86; Antonopoulos et al., '87) as a small nucleus situated at the lateral aspect of the external capsule, ventrolaterally to the striatum. In the present study, we describe the anatomy of the hedgehog claustrum, the morphology of its constituent neurons, and the efferent connections with the cortical and subcortical structures. This species has been studied by Druga ('74a), and the morphology of its neuronal constituents, and the efferent connections with the thalamus (Trojanowski and Jacobson, '75; Sloniewski et al., '85; Carey and Neal, '86), the pretectum (Sloniewski et al., '85), the putamen (Druga, '72; Flindt-Egebak and Olsen, '78), medial septal nuclei (Spector et al., '74), the locus coeruleus (Pickel et al., '74), the hypothalamus (LeVay and Sherk, '81a), as well as with limbic areas (Markowitsch et al., '84; Willhite et al., '86) have been described. These studies have suggested that the claustrum, through its reciprocal neocortical and limbic connections, participates in the integration of sensory, motivational, emotional, and mnemonic information.

Despite the accumulating findings in other species, particularly the cat, information about the cytology of the claustrum and its connections in the hedgehog brain is essentially absent. The normal anatomy of the claustrum in this species has been studied by Druga ('74), and the claustrum has been noted by others (Valverde and Facal-Valverde, '86) and in our previous papers (Papadopoulos et al., '86; Antonopoulos et al., '87) as a small nucleus situated at the lateral aspect of the external capsule, ventrolaterally to the striatum. In the present study, we describe the anatomy of the hedgehog claustrum, the morphology of its constituent neurons, and the efferent connections with cortical and subcortical structures.

MATERIALS AND METHODS

Twenty-six adult hedgehogs of either sex were used in this study. Hedgehogs were caught from the wild, mostly during their active period, and kept in a cage with food and water only for the minimum time required to perform the experiments.

Golgi-impregnated material

Seven hedgehogs were used to obtain the Golgi-impregnated material. Five anesthetized hedgehogs were perfused through the heart, with 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M phosphate buffer (PB, pH 7.4). The brains were removed and stored in the same fixative for one or two days. Then blocks of the hemispheres, 5–6 mm thick, containing the claustrum, were stained with the rapid Golgi method. Briefly, blocks were placed in a solution containing 3% potassium dichromate and 0.33% osmium tetroxide in distilled water for seven days. They were then briefly washed in 0.75% silver nitrate and immersed in a fresh solution of 0.75% silver nitrate for 48 hours. These blocks were then shelled in paraffin, and cut in the coronal plane into sections 80–100 μm thick on a sliding microtome. Sections were collected in absolute alcohol, and taken through absolute alcohol, methyl salicylate, and xylene with two changes in each. Finally, they were placed on slides, blotted with filter paper and coverslipped with Permount. Every fourth section was stained with toluidine blue for purposes of orientation and more accurate localization of the Golgi-stained neurons.

Another two hedgehogs were decapitated under ether anesthesia, and their brains were removed and placed in Golgi-Cox solution prepared according to Van der Loos ('59). When impregnation appeared complete on trial sections (3–4 weeks), they were embedded in collodion and sectioned coronally into sections 160 μm thick on a sliding microtome. Sections were then passed through 70% and 50% alcohol to 28% (NH₄)OH: distilled water (2:1) for 5 minutes, and then to 28% (NH₄)OH: distilled water (1:1) for 25 minutes. They were subsequently transferred into 1% Na₂S₂O₅ for 7 minutes and then taken through an ascending series of alcohols to butanol: absolute alcohol (1:1) for 5 minutes. The sections were finally cleared in histoclear, mounted with histomount, and coverslipped.

In addition, three anesthetized hedgehogs were perfused with saline followed by 10% formaldehyde in 0.1 M PB and their brains were stained with the Nissl or the Kluver-Barrera methods.

In our analysis of the material, the claustrum and the neighbouring areas, as well as Golgi-impregnated neurons within the claustrum, were drawn with the aid of a drawing tube attached to a microscope. In the drawings, an unambiguous boundary is shown by a continuous line, while a broken line indicates the best estimate of an uncertain boundary.

Horseradish peroxidase experiments

A total of 16 hedgehogs were used for this series of experiments. All animals received injections of either a 4% wheat germ agglutinin-horseradish peroxidase (WGA-HRP; Sigma) or a 30% HRP (Sigma) solution in sterile water. Eleven hedgehogs each received 0.1 μl WGA-HRP injections in various neocortical sites, and 5 animals each received 0.2 μl of the HRP or WGA-HRP solution into the thalamus. Animals were anesthetized with flurothyl administered in an open system and then were immobilized on a stereotactic apparatus. The brain was removed from an intact skull, and a small craniotomy, using a dental drill. Small pricks in the pia were made with a fine needle under microscopic guidance to avoid damage of blood vessels or the brain. Coordinates for the thalamic injections were estimated in two fixed brains used for another study. All injections were made over a period of 10 minutes with a 1-μl Hamilton microsyringe or with a glass micropipette attached to the microsyringe. In each case, the injection needle was left in place 10 minutes, both before and after the injection. Animals were allowed to survive for 24 hours, after which they were anesthetized with ether and perfused through the heart with 300 ml of saline followed by 450 ml of a mixture of 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M PB. The brains were removed and immersed in the same fixative with 5% sucrose for approximately 5 hours and then placed in 30% sucrose in PB overnight. Serial frozen sections were cut in the coronal plane at 40 μm and every other section was collected. Sections were then treated according to the HRP histochemical methods of Mesulam ('78) or Olucha et al. ('85). Finally, the sections were mounted on gelatin-coated slides, counterstained with 1% neutral red, rapidly dehydrated, cleared in xylene and covered with Permount.

The distribution of WGA-HRP-labeled cells in the claustrum and the neighbouring cortical areas was plotted with the aid of a drawing tube attached to a microscope. Draw-
The claustrum is a prominent structure in the hedgehog brain, with a total rostrocaudal extent of about 6 mm. It extends caudally from about 2 mm from the frontal pole to about mid-amygdaloid body levels (Fig. 1). In the hedgehog, as in other animals, two divisions of the claustrum can be distinguished in both Nissl and Klüver-Barrera stained sections: the dorsal and the ventral claustrum. The dorsal claustrum is more extensive below the orbital sulcus, the sole sulcal depression in the otherwise smooth surface of the neocortex. In transverse sections, the dorsal claustrum appears as an inverted “drop-shaped” mass of neurons that lies lateral to the white matter, dorsomedial to the rhinal sulcus, and dorsolateral to the striatum. Ventrolaterally, it merges with the ventral claustrum. The approximate juncture of the two divisions lies medial to the piriform cortex-neocortex transition area, at the level of the rhinal sulcus. However, the boundary between dorsal and ventral claustrum cannot be distinguished with certainty in normal stained sections. Rostrally, the dorsal claustrum shares a border with the frontal cortex, whereas caudally it diminishes in size at the level of the amygdaloid body and it grades into the posterior insular cortex. The dorsal claustrum can be divided into lateral and medial parts by a thin sheet of fibers and some neurons. This sheet is wider rostrally but gradually disappears caudally. The medial part is a sheet of cells adjacent to the white matter and in continuity with layer VI dorsally and with the ventral claustrum ventrally. We include this sheet of cells as part of the dorsal claustrum owing to its close proximity to it and based on previous reports (Druga, ’74; Valverde and Facal-Valverde, ’86) that describe this sheet as the dorsal claustrum. However, our WGA-HRP experiments and the structure’s resemblance with cortical layer VI, suggest that this sheet may be a ventral continuation of this layer (see below). The lateral part of the dorsal claustrum is considerably larger and merges with the cerebral cortex; in this paper, the lateral part will be referred to simply as dorsal claustrum. The dorsal claustrum is poorly separated from neocortical layer VI because of the lack of an apparent extreme capsule. However, the neocortex immediately above the rhinal sulcus clearly consists of the usual six layers and can be differentiated, although with difficulty, from the dorsal claustrum. Neocortical layers I and II, which are particularly thick, as well as layer III, extend over the corresponding layers of the piriform cortex. Neocortical layer IV terminates at about the level of the rhinal sulcus. Neocortical layers V and VI merge at the lateral aspect of the dorsal claustrum. They terminate mainly above the rhinal sulcus but a few cells continue ventrally at the lateral aspect of the juncture of the dorsal and ventral claustrum.

The ventral claustrum, slightly larger than the dorsal, resembles a triangle in cross-section. Its medial half shares a border with the external capsule. More caudally, it is located between the lateral amygdaloid nucleus and the piriform cortex. Although the extreme capsule is not apparent, the ventral claustrum is distinguished from the third layer of the piriform cortex by the greater density of neurons in the ventral claustrum. The rostral pole of the ventral claustrum is poorly defined and blends with the anterior olfactory nucleus. Caudally, the ventral claustrum tapers until, at about mid-amygdaloid body levels, it is no longer identifiable.

Neuronal types

In Nissl and Klüver-Barrera stained sections, neurons in the dorsal claustrum appear as large multipolar cells, among which fewer spindle-shaped cells are dispersed. In general, there is no orderly alignment of cells like that of the cerebral cortex. Neurons in the ventral claustrum are slightly smaller and less densely aggregated than those in the dorsal claustrum. As Nissl and Klüver-Barrera preparations do not stain dendrites, detailed morphological information was obtained from Golgi-stained sections.

The absence of pyramidal cells in the dorsal claustrum distinguishes it from the cerebral cortex, when Golgi-stained sections are viewed under low magnification. Pyramidal cells, which dominate the mammalian neocortex, typically possess a prominent apical dendrite extending towards the pial surface. This radial arrangement of the apical dendrites is absent in the dorsal claustrum. Impregnated neurons within the dorsal claustrum can be divided into two main types: (1) large multipolar cells with spiny dendrites distributed throughout the nucleus, and (2) multipolar cells with aspiny beaded dendrites that are fewer in number and dispersed among the spiny cells. It should be mentioned that the aspiny cells are less frequently observed in the Golgi-Cox than in the rapid Golgi material. Similar neuronal types are also found in the ventral claustrum and therefore, the descriptions given below apply to neurons in both parts of the nucleus.

Spiny neurons. Spiny neurons are the most frequently impregnated in both the Golgi-Cox and the rapid Golgi material (Fig. 2). They are large in size with a long axis of 20–30 μm. On the basis of soma shape and number of primary dendrites, three subtypes can be distinguished: neurons with a polygonal soma and 4–7 primary dendrites; neurons with a triangular soma and typically 3–5 primary dendrites; and fusiform neurons with 2–4, usually 2, primary dendrites emanating from the poles of the soma. In all subtypes, primary dendrites gradually taper distally, giving rise to several branches. Primary dendrites and their branches show no apparent preferential direction and in most cases, they do not extend beyond the boundaries of the claustrum. In a few cases, they are observed to enter cortical layer VI or the white matter. However, they typically have a very extensive dendritic field with dendrites that span 100–300 μm. Cells lying near the white matter tend to have a fusiform soma and dendrites running parallel to the fibers of the white matter. The most prominent feature of these cells is the large number of spines. Spines are fewer or absent in proximal parts of primary dendrites, but they are numerous in their distal parts, and in the secondary and tertiary dendrites. Many spines show a slender stalk and a bulbous terminal enlargement, whereas others have a longer stalk and an elongated enlargement.
Fig. 1. Drawings of coronal sections in which the claustrum of the hedgehog is depicted throughout its rostrocaudal extent (a-f). Scale bar = 2 mm. Abbreviations used in this and the following figures: ac, anterior commissure; CP, caudate/putamen; dcl, dorsal claustrum; IC, internal capsule; LV, lateral ventricle; OrS, orbital sulcus; RS, rhinal sulcus; TH, thalamus; vcl, ventral claustrum; WM, white matter; X, nucleus X; I–VI, respective cortical layers.
Fig. 2. Drawings showing spiny cells of the dorsal (a, b, e) and ventral (d) claustrum and the morphology of their spines (e). Aspiny cells of the dorsal claustrum are shown in f and g. Scale bar = 100 μm; in e = 30 μm.

Aspiny neurons. Aspiny neurons represent a small proportion of the impregnated neurons in the rapid Golgi, and particularly in the Golgi-Cox material (Fig. 2). They are large multipolar cells with a long axis of 20–30 μm and 3–7 primary dendrites, which only occasionally give off second-order dendrites. Among them are some cells with smaller fusiform, ovoid or round somata and fewer dendrites. The main morphological characteristics of these cells are the
sparserness of spines and the beaded appearance of their dendrites. Dendrites show no apparent preferential orientation and they do not extend beyond the claustrum. Their dendritic field is less extensive than that of the spiny neurons. Axons are rarely impregnated, and when they are, they are stained only for short distances and have never been seen to leave the claustrum.

**Horseradish peroxidase experiments**

*Injections in neocortical areas.* In these experiments, eleven hedgehogs received WGA-HRP injections in various neocortical areas. Two injections were placed in the frontal cortex, 3 in the motor cortex, 3 in the somatosensory cortex, 1 in the auditory cortex and 2 in the visual cortex. In the majority of these injections, there was a moderate diffusion of the enzyme into neighboring cortical areas, because the WGA-HRP injections were relatively large in order to produce a substantial number of retrogradely labeled cells in the claustrum. The injection sites were identified with the help of the cytoarchitectonic atlas of Brodmann ('09), the works of other investigators (Lende and Sadler, '67; Hall and Diamond, '68; Kaas et al., '70; Gould et al., '78; Valverde et al., '86), as well as our stained normal and experimental sections. In addition, examination of sections through the thalamus showed retrogradely labeled neurons in thalamic nuclei known, from studies in the hedgehog (Hall and Diamond, '68; Killackey and Ebner '72; Gould et al., '78; Valverde et al., '86) and other animals, to project to specific cortical areas. Thalamic nuclei were identified in Nissl and Klüver-Barrera stained series of hedgehog brains as well as with the help of the outlines presented by other authors for this animal (Erickson et al., '67; Killackey and Ebner, '72; Gould et al., '78).

*Injections in the prefrontal area.* Previous anatomical and physiological studies have shown that there is very little, if any, cortex between sensory and motor areas of the hedgehog brain. However, two of our injections, placed in the rostralmost part of the neocortex, labeled cells only in the dorsomedial (DM) and medioventral (VM) thalamic nuclei, known from studies in other species to project mainly to the prefrontal cortex. None of the "motor" nuclei in the thalamus is labeled. Following such injections, labeled cells are found in the dorsal claustrum of both sides; however, they are much more numerous in the ipsilateral than in the contralateral side (Figs. 3, 4). Labeled neurons in the ipsilateral side are found in the whole rostrocaudal extent of the nucleus, slightly closer to its lateral margin. Only a few labeled cells are situated medial to it, and very few are present in the ventral claustrum. Slight anterograde labeling is evident, in all experiments, with injections in various neocortical areas. A substantial number of labeled cells is also found throughout cortical layers II–VI above the rhinal sulcus, as well as in the depth of this sulcus. In the side contralateral to the injection, labeled cells have similar location to those of the ipsilateral side, with the exception of the area medial to the dorsal claustrum, where no labeled cells are found. Numerous labeled cells are also found in cortical layers II–IV, mainly in areas corresponding to the injection site. Fewer cells are present in layers V and VI just above the dorsal claustrum (Fig. 4).

*Injections in the motor area.* Included in this group are injections in areas 4 and 6 of Brodmann ('09), characterized mainly by the presence of thick layers V and VI. These injections result in labeling of the "motor" nuclei of the thalamus, namely the ventral lateral (VL) and ventral anterior (VA). Other nuclei that contain labeled cells are the paracentral (PC) and central lateral (CL), midline nuclei and the DM. Labeled cells are also found throughout the dorsal claustrum of both hemispheres (Fig. 5). Fewer cells are found in layers II–VI and in the depth of the rhinal sulcus, but only at posterior levels. In the example given in Figure 5, the injection is located caudal and medial to the injection shown in Figure 3 (injection in the prefrontal area). In this case, labeled neurons are located closer to the medial margin of the dorsal claustrum than in Figure 5, but as in all other cases, there is a substantial degree of overlap. Very few cells are found scattered medial to the dorsal claustrum on the ipsilateral side.

*Injections in the somatosensory area.* Three injections were placed in the somatosensory area (areas 5 and 7 of Brodmann, '09). In this area, layers III and IV are more densely populated than in the motor cortex. Labeled cells in the thalamus are found mainly in the ventral posterior (VP) and in PC, CL, posterior complex (PO), VM, and midline nuclei. However, depending on the injection site, a few labeled cells are also found in VL, VA, dorsal lateral geniculate nucleus (dLGN) and lateral posterior nucleus (LP). These findings show that the above injections are placed mainly in the somatosensory area but that there is also diffusion of the enzyme either into the motor or the visual area. However, irrespective of the exact site of the injection, labeled cells, in all cases, are found again throughout the dorsal claustrum, mainly in the ipsilateral side (Figs. 6, 7). A considerable number of labeled cells are also found in the cortex, deep into the rhinal sulcus of both sides (Figs. 6, 7). In the example shown in Figure 11, the injection is located in the lateral part of the somatosensory cortex and covers the depth of the rhinal sulcus and part of the ventral claustrum and the piriform cortex. Numerous labeled cells, in this case, are found in all rostral levels of the ipsilateral dorsal claustrum. Contralaterally, they are found throughout the nucleus and in the depth of the rhinal sulcus at levels corresponding to the injection site (Fig. 6). No labeled cells are found in the ventral claustrum. Within the dorsal claustrum, labeled cells are located closer to its lateral margin, particularly at rostral levels.

*Injections in the auditory area.* One injection was placed in the auditory area (areas 20–22 of Brodmann, '09). This injection extended to the cortex in the depth of the rhinal sulcus and to the ventral claustrum. Labeled cells in the thalamus are found mainly in the medial geniculate nucleus (MGN), but a few cells are also found in the dLGN and LP, suggesting minor spread of the enzyme into the visual cortical area. Labeled cells are found in the dorsal claustrum, bilaterally and, as in experiment cl.554 (Fig. 6), in the cortex deep into the contralateral rhinal sulcus at levels corresponding to the level of the injection site. No labeled cells are found in the contralateral ventral claustrum.

*Injections in the visual area.* Two injections were placed in the visual area (Fig. 8). The visual area is characterized by the thickness of layer IV, as well as by a decrease in the density of cells in layer VI. Retrogradely labeled neurons in the thalamus are found mainly in the dLGN as well as in the LP. Following such injections, labeled cells are found throughout the rostrocaudal extent of the dorsal claustrum of both sides, but are considerably less numerous in the contralateral side. No labeled cells are found medial to the claustrum. A few labeled cells are also found in the depth of the rhinal sulcus at caudal levels.
Injections in the thalamus. Five hedgehogs received WGA-HRP or HRP injections in the thalamus. Injections were placed either rostrally (3 animals) or caudally (2 animals).

The injections in the rostral half of the thalamus stained all thalamic nuclei at this level, including most parts of the ventral anterior (VA) and ventral lateral (VL) nuclei, caudal parts of the DM, and the LGN, without apparent diffusion of the enzyme into hypothalamic areas. Caudally, the injection extended to the VP and to a small part of the pretectum (Fig. 9). Retrogradely labeled cells are found throughout the ipsilateral dorsal claustrum and are located...
Fig. 4. Drawings of coronal sections showing the distribution of labeled cells in the contralateral hemisphere following the injection shown in Figure 3. Scale bar = 2 mm.

more ventrally and caudally than labeled cells after cortical injections. Five to ten labeled cells are found in each section. On the contrary, nearly all cells in layer VI and a substantial number of neurons in layer V of the neocortex are labeled. Numerous labeled cells are also found medial to the dorsal claustrum. They are in continuity with labeled cells in layer VI and show no morphological differences from the latter. No labeled cells are found in the ventral claustrum.

The injections in the caudal half of the thalamus stained all thalamic and hypothalamic nuclei at this level, as well as the cerebral peduncle, the substantia nigra, and the ventral tegmental area. Caudally, the injection covered part of the pretectum, the midbrain tegmentum, and the medial part of the MGN. The VP was stained throughout its extent, while caudal parts of the VA and VL were also involved. Rostral parts of the hypothalamus were not stained (Fig. 11a). Retrogradely labeled cells show the same distribution, both in the claustrum and in the neocortex, as with injections that stained the rostral half of the thalamus (Fig. 10). However, labeled cells in layer V are much more numerous. Caudally, labeled cells in layers V and VI gradually occupy the region where, in more rostral levels, the dorsal claustrum is located (Figs. 10f, 11d). Here, neurons are pyramidal in form with clear apical dendrites; they differ from those in the dorsal claustrum in their morphology, orientation and distribution pattern (compare Figs. 10e, 11c,b with Figs. 10f, 11d). This area obviously belongs to the posterior insular cortex.

Following WGA-HRP injections in the thalamus, anterograde labeling is evident in layer IV and lower part of layer III (Figs. 10a–d arrows) but is never observed in the dorsal claustrum.

DISCUSSION
Anatomy of the claustrum

Origin of the claustrum. The claustrum is usually described as a telencephalic structure separated from the striatum by the external capsule, and from the cerebral cortex by the extreme capsule. On the basis of the proximity of the claustrum to the insular cortex and the appearance of
its cells in Nissl stained sections, early investigators (Brodmann, '09) suggested that this nucleus is the innermost part of layer VI, which is differentiated as a separate layer in animals such as primates and cat, but is not separated completely from layer VI in the rat and rabbit. However, developmental studies have shown that cells that constitute
the claustrum are not closely associated with any region of the neocortex. Instead, the pattern of cell migration and the position of the embryonic claustrum appears to be unique (Filimonoff, '66). In addition to these ontogenetic differences, there is also evidence which suggests that the insula and the claustrum differ in functional specialization. For example, anatomical and physiological studies in the cat revealed considerable involvement of the claustrum in visual function (Olson and Graybiel, '80; LeVay and Sherk, '81b; Sherk and LeVay, '81, '83). In contrast, unit recordings in the insula show that visual responses are less common than responses to other sensory modalities (Suda-

Fig. 7. Drawings of coronal sections of the right hemisphere showing an injection in the somatosensory (e) and partly in the visual (lower right) cortex as well as the distribution of labeled cells in the same hemisphere in experiment cl.538 (rostrocaudal sequence a–e). Scale bar = 2 mm.

Fig. 6. Drawings of coronal sections of the right hemisphere showing the injection site in experiment cl.554 (drawings at top) and the distribution of labeled cells in the contralateral hemisphere (rostrocaudal sequence a–f). Scale bars = 2 mm.
Fig. 8. Drawings of coronal sections of the right hemisphere showing an injection in the visual cortex (lower right) and the distribution of labeled cells in the same hemisphere in experiment cl.523 (rostrocaudal sequence a–e). Scale bar = 2 mm.
kov et al., '71). The present findings suggest that in the hedgehog, too, the claustrum is distinct from the neocortex. According to our description, the cerebral cortex immediately dorsal to the rhinal sulcus, which has been reported by Brodmann ('09) as the insular cortex, has all six layers characteristic of neocortical areas and contrasts to the cellular appearance of the claustrum. The cellular composition and connectivity of the claustrum further support this...
Fig. 10. Labeled cells in cortical layers V and VI and the dorsal claustrum of the left cerebral hemisphere in experiment cl. 573 following the injection in the thalamus shown in Figure 11a. Arrows indicate anterograde labeling within cortical layer IV and lower part of layer III. Sections a-f indicate rostrocaudal sequence. Scale bar = 1 mm.
Point of view. There is one type of cell, the spiny multipolar neuron, dominating this structure, in contrast to any neocortical area in the hedgehog (Shkol'nik-Yarros, '71; Valverde and Facal-Valverde, '86; present observations) or in another insectivore, the common european mole (Ferrer, '86).

The above data provide little reason to consider the claustrum as part of the cerebral cortex. Therefore, what we describe here as dorsal claustrum could be neither an "invagination of the frontal pole" (Valverde and Facal-Valverde, '86) nor "the transitional neo-paleocortical area" (Druga, '74). We still feel uncertain about the small medial part of the dorsal claustrum which has been described by others (Druga, '74; Valverde and Facal-Valverde, '86) as the dorsal claustrum. However, based mainly on its connectivity pattern, we classify it as a ventral continuation of cortical layer VI. Regarding the ventral claustrum, our present description is in accordance with that of Druga ('74) and at variance with those reports (Papadopoulos et al., '86; Valverde and Facal-Valverde, '86; Antonopoulos et al., '87) that identify as the claustrum a small nucleus (nucleus X, Fig. 1), situated at the lateral aspect of the external capsule, ventrolateral to the striatum. The following points support our view: (1) in all species examined, including the hedgehog, the claustrum has been described as a structure consisting of a dorsal part lying above the rhinal sulcus and a ventral part lying below it. In contrast, nucleus X lies at a considerable distance from the rhinal sulcus; (2) neurons in the ventral claustrum show only minor differences from those in the dorsal claustrum. However, neurons within nucleus X are much smaller, and mainly round or fusiform in shape, and have fewer dendrites and more restricted dendritic fields; (3) the dorsal claustrum has connections with neocortical areas, whereas the ventral claustrum has connections with paleocortical areas (Druga, '71; Markowitsch et al., '84). However, following neocortical injections of HRP no labeled cells were found in nucleus X; (4) finally, in the hedgehog the rhinal sulcus separates the small neocortex from the much larger paleocortex. Therefore, a relatively large ventral claustrum would be expected rather than a small area such as nucleus X.

Cell types. Analysis of our Golgi material suggests the presence of two main types of neurons in the claustrum of the hedgehog: the spiny multipolar and the aspiny multipolar neurons. Although three subtypes of the spiny neurons may be distinguished, they are all presumably projection neurons. Electron microscopic degeneration studies (Jurandie et al., '71; LeVay and Sherk, '81a; Hinova-Palova et al., '88) have shown that the majority of corticothalamic terminals contact spines and form asymmetrical synapses. The prevalence of terminations on spines suggests that the spiny neurons receive the bulk of the cortical input. The HRP experiments described in this, as well as in other studies (Olson and Graybiel, '80; LeVay and Sherk, '81a; Sloniewski et al., '86), have shown that, following injections in some cortical areas, nearly all neurons within a region of the claustrum are retrogradely labelled, suggesting that most cells in the claustrum project back to the cortex. It seems possible, therefore, that the corticothalamic loop involves only one synapse in the claustrum. However, the possibility that one or more claustral interneurons are included in this loop cannot be excluded.

The claustrum was found to project to brain structures in addition to the cortex, such as the thalamus (Trojanowski and Jacobson, '75; Sloniewski et al., '85; Carey and Neal, '86; present findings), the pretectum (Sloniewski et al., '85), and putamen (Druga, '72; Findt-Egebak and Olsen, '78). Therefore, axons of spiny neurons either undergo extensive collateralization, or subpopulations of spiny neurons that project to different brain structures exist. LeVay and Sherk ('81a) found that aspiny cells receive a lesser portion of the cortical input onto their beaded dendrites than that received by the spiny cells. In our preparations, aspiny neurons, showing only few spines, constitute a minor proportion in the claustrum. We could not assign, with confidence, these neurons as either projection neurons or interneurons, since their axons were either not impregnated at all or were impregnated for only short distances. However, in all species examined (cat: LeVay and Sherk, '81a; Hinova-Palova, '86, primates: Brand, '81, man: Braak and Braak, '82, Spahn and Braak, '85) aspiny neurons have been suggested to be interneurons. The possibility, however, that the larger multipolar aspiny cells in our preparations are also projection neurons, cannot be excluded. The beaded appearance of dendritic processes of many of these cells has also been reported for large aspiny neurons in the monkey claustrum (Brand, '81), while in the human claustrum, large aspiny neurons show no varicosities (Braak and Braak, '82).

Our description of two types of neurons in the hedgehog claustrum is consistent with Brand's ('81) classification for the primate claustrum. Brand ('81) describes three types of neurons, but only one, the large spiny cell, is classified as an efferent neuron. These similarities to the hedgehog claustrum suggest that the claustrum undergoes only minor alterations during phylogensis. However, other investigators describe more neuronal types in the claustrum of both the cat (Hinova-Palova, '86) and man (Braak and Braak, '82; Spahn and Braak, '85).

Connections of the claustrum

The present HRP experiments show that claustral projections are distributed to the entire neocortex. Regardless of the location of the injection site, claustrocortical projections arise in the dorsal claustrum. The finding of few labeled cells in the ventral claustrum following injections in the prefrontal cortex is in conformity with observations in the cat (Riche and Lanoir, '78), showing minor projections from the ventral claustrum to neocortical areas. However, this finding, at least for the hedgehog, does not contradict the generally held view that the dorsal claustrum is connected with neocortical areas, whereas the ventral claustrum is connected with paleocortical areas (Druga, '71; Krettek and Price, '77a,b; Markovitsch et al., '84). Labeled cells in our experiments were distributed in the same region on both sides, with a clear-cut ipsilateral preference. Nevertheless, labeled cells in the contralateral claustrum were present in every case, regardless of the injection site. This result agrees with those from other species (Riche and Lanoir, '78; Macchi et al., '83; Neal et al., '86). Only a small proportion (fewer than 1% of the total population of labeled cells) of the claustral neurons that project to the cortex have bihemispheric axonal collaterals (Minciacci et al., '85; Dreher et al., '90). Other studies have shown, moreover, that the same regions of the ipsilateral and contralateral claustrum appear to be the convergence site of fiber pathways from homotopic cortical areas of both hemispheres (Squatrito et al., '80a,b).

The present findings show that, in the hedgehog, certain cortical regions such as the prefrontal, motor and somato-
sensory areas, are innervated by a higher number of claustral fibers than others, namely the auditory and visual areas. Since the hedgehog shows only two visual areas, the fewest of any mammal yet mapped (Gould and Ebner, '78), it is pertinent that one of the least differentiated visual systems. This lack of differentiated visual cortex may account for the limited claustrocortical connections with the visual areas. However, quantitative comparisons cannot be made, since our injections were not equal in size. In addition, claustrocortical connections with the auditory cortex are present in the hedgehog, as has also been shown in the cat (Macchi et al., '83; Neal et al., '86; Hinova-Palova et al., '88).

Multiple sets of nonthalamic subcortical projections reach the neocortex directly, in addition to those arising in the claustrum. Among them, are those that arise in the basal forebrain and in serotonergic, noradrenergic, and dopaminergic cell groups of the brain stem. These sets of nonthalamic afferents display different patterns of cortical distribution, but they also display the common feature of being widely distributed within the neocortex. Comparisons of the present results with previous findings concerning the basal forebrain projections to the cortex in the hedgehog (Dinopoulos et al., '88) suggest that claustral projections show a higher degree of topographical organization. However, claustral projections are only roughly topographically organized and cells projecting to different cortical areas are intermingled throughout the rostrocaudal extent of the dorsal claustrum. Thus, compared to the dorsal thalamic projections to cortex, the claustral projections are widespread not only in the hedgehog, but also the cat (Macchi et al., '81, '83; Drugs, '82; Pearson et al., '82; Markowitsch et al., '81, '83; Druga, '82; Pearson et al., '82; Markowitsch et al., '86). On the other hand, widespread projection does not mean that the cells are not segregated according to their connections. In fact, experiments in the cat (Norita, '83; Macchi et al., '83) and in the rat (Sloniewski et al., '86) suggest that individual neurons in the claustrum project only to one cortical area so that, even though claustral cells projecting to different cortical areas are intermingled, an individual cell may have specific projections. A similar issue concerning the specificity of connections is raised by the observation that at least five visual cortical areas in the cat are superimposed retinotopically in the claustrum (LeVay and Sherk, '81a). The above authors concluded that single claustral cells are in a position to receive input from multiple visual cortical areas, but whether they actually do so remains unknown. In addition, Pearson et al. ('82) found that in the monkey, restricted parts of the claustrum may project to widely separated but interconnected areas of the cerebral cortex. This seems to be the case in the cat as well (Markowitsch et al., '84).

It has been shown that in all species studied so far (rat: Miller and Vogt, '84; Shammem et al., '84; Sloniewski and Pilgrim, '84; Carey and Neal, '85; Sloniewski et al., '86; Dreher et al., '90; cat: Chadzypapanagiotis and Narkiewicz, '71; Jayaraman and Updyke, '79; Sanides and Buchholtz, '79; Irvine and Brugge, '80; Olson and Graybiel, '80; Squitrito et al., '80a, b; LeVay and Sherk, '81a; Macchi et al., '81; Drugs, '82; Adensro and Cavene, '86; Guldin et al., '86; primates: Kunze and Akert '77; Pearson et al., '82; Tiggas et al., '82, '83; Doty, '83; Weber and Yin, '84) the claustrum is reciprocally connected with the cortex. In the hedgehog, surprisingly, only minor anterograde labeling was found in the claustrum following cortical WGA-HRP injections, although anterograde labeling was clearly evident in thalamic nuclei. Possible explanations are: (1) in the hedgehog, the corticothalamic fibers are less numerous than in other animals; (2) some labeled terminals have faded because of technical factors; and (3) labeled terminals may be covered by the strong retrograde labeling.

The claustrocortical and corticothalamic connections have been shown to be organized also by cortical layer. Thus, claustrocortical terminals in the tree shrew are more numerous in layer IV and less numerous in layer IIIb, VI and I of the striate cortex (Carey et al., '80). The claustrum receives a projection from layer VI of the striate cortex (Carey et al., '80). This is also the case in the cat (Olson and Graybiel, '80; LeVay and Sherk, '81a; LeVay, '86) and may reflect an organization common to all mammals. In addition, it has been shown in the cat that cells in layer VI of the striate cortex that project to the claustrum are distinct from those projecting to dLGN (Gilbert and Kelly, '75; LeVay and Sherk, '81a). There is also evidence that claustral efferents provide a major input to local circuit cortical neurons, which may be inhibitory in function (LeVay, '86). These findings, in conjunction with electrophysiological evidence (Pito and Lassonde, '81; Tsumoto and Suda, '82; Sherk and LeVay, '83), suggest that the claustrum is capable of exerting an important influence on the input to cortex. It has been suggested that this influence may be altered by subcortical input to the claustrum arising, particularly, in thalamic nuclei reciprocally connected with the claustrum (Sloniewski et al., '85; Carey and Neal, '86). The present results do not support this view. Although a claustrothalamic projection was clearly demonstrated, there was little evidence for a thalamoclustral projection. In contrast, retrograde transport studies indicate that claustrothalamic cells are numerous in several species studied (Sloniewski et al., '85; Carey and Neal, '86; Hinova-Palova, '86). It appears that the claustrocortical and claustrothalamic cells are intermingled throughout the claustrum. This is the case in the hedgehog as well; however, claustrothalamic cells are much less numerous and are found in more ventral and caudal parts of the nucleus, whereas claustrocortical cells are located in more dorsal and rostral parts. In addition, unlike the claustro cortical and cortico claustral connections, the claustrothalamic connection is only ipsilateral.

The cerebral cortex and the thalamus are not the only targets of the efferent claustral axons. The findings that the claustrum projects also to the neostriatum, the pretectum, the zona incerta, and the peripeduncular nucleus (Dreher et al., '72; Flintd-Engehuk and Olson, '79; Carey and Bean, '81; Sloniewski et al., '85) suggest an extensive distribution of claustral efferents.

Concluding remarks

The claustrum is a telencephalic structure with widespread reciprocal connections with the neocortex and, therefore, may regulate or integrate the cortical processing of information in many sensory and motor domains. However, the functional role of these interconnections is, at present, poorly understood. Specific suggestions have been made for the visual cortex (Sherk and LeVay, '83). In terms of its reciprocal connections with the neocortex, it bears a
resemblance to the thalamus; however, since there are no major ascending projections to the claustrum, it does not share the relay function of the thalamus and instead, has been suggested to play a satellite role for the neocortex (Olson and Graybiel, '80). With respect to the hedgehog, it has been suggested that this species possesses a small neocortex and shows a primitive stage of cortical organization (Valverde and facial-Valverde, '86). A similar description might be expected to apply to the hedgehog claustrum, since the size of this structure seems to increase proportionally with cortical volume (Sherk, '86). However, the present findings show that the hedgehog possesses a very well developed dorsal claustrum, which is connected with all major neocortical areas and with the thalamus. This suggests that the claustrum in the hedgehog shares the same pattern of relationship with other forebrain structures, as is found in mammalian species with more highly developed cortices.

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LITERATURE CITED

THE CLAUSTRUM OF THE HEDGEHOG


Sudakov, K., P.D. MacLean, A. Reeves, and R. Marino (1971) Unit study of exteroceptive inputs to claustrocortex in awake, sitting, squirrel monkeys. Brain Res. 28:10-34.


