PRETECTAL CONNECTIONS TO THE CLAUSTRUM: AN HRP RETROGRADE TRANSPORT STUDY IN CATS

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Abstract. In 28 cats after injections of HRP solution (0.20–0.50 μl) to the claustrum from the lateral side or from above, large numbers of HRP labeled cells were found in the anterior pretectal nuclei. This concerned the anterior compact nucleus more than the anterior reticular. This type of labeling was not observed after injections of HRP to the cerebral cortex and striatum. The results speak in favor of a great number of pretecto-claustral axons connecting the anterior pretectal neurons with the claustrum.

INTRODUCTION

The claustrum is situated between the cerebral cortex and the striatum on the border of the paleo- and neocortex. Its connections were unknown up to the ’60s when it was found that it is connected with large areas of the cortex by means of cortico-claustral and claustro-cortical axons (7, 12, 32). It was also found (28, 36), that the claustrum is connected with the cerebral cortex not only ipsilaterally, but also with the cortical areas of the other hemisphere. The cortico-claustral connections were confirmed both by electron microscopic (25, 35) and electrophysiological studies (21).

The axons connecting the claustrum with the cerebral cortex are organized topographically (12–14, 32, 33, 36). The sensori-motor cortex possesses connections mainly with the anterior part of the claustrum, while the visual cortex with the posterior part to which the auditory
cortex is also connected. Contrary to the corticoclaustral relations not much is known about the connections of the claustrum with subcortical areas. Rosegay (41) described degeneration of nerve cells in the posterior part of the claustrum of the cat following lesions in the mesencephalon and pons. Hiddema and Droogleever-Fortuyn (22) found degenerated fibres approaching the claustrum as a result of a thalamic lesion. Ruiz (43) on damaging the claustrum observed degenerating nerve fibres running to the medulla oblongata. Droogleever-Fortuyn and Stefens (11) basing on observations of the Vogts (54) and of McLardy (31) on human pathological material, found that there are connections between centrum medianum and the claustrum. Connections of the claustrum with the thalamus were also described by Nauta and Whitlock (34) as well as by Starzl (49). Pickel et al. (39) when studying the efferent connections of locus ceruleus found axons reaching the claustrum. However, none of the above mentioned possible connections seem to be the main source of subcortical (37) input to the claustrum. Some studies even suggest that claustrum does not receive other than corticofugal connections. Therefore the aim of our studies was to determine which subcortical nuclei send axons to the claustrum. A pilot study was carried out which showed the possibility of numerous fibres running from the pretectal area to the claustrum. Our present investigations were an attempt to answer the following questions:

1. Does the pretectal area contain neurons which axons terminate in the claustrum?
2. If so, in which nuclei of the pretectal area do the above axons have their origin?
3. Are the presumed connections of the pretectal area with the claustrum so numerous as to be the main source (or one of the main sources) of information sent from the lower levels of the central nervous system to the claustrum?

THE STRUCTURE OF THE CLAUSTRUM AND THE PRETECTAL AREA

The claustrum appears in all mammals as a structure more or less separated from the cortex. The claustrum of the cat is a large area of the telencephalon stretching rostro-caudally over a length of about 12–14 mm. It possesses two main parts: the dorsal and the ventral. The dorsal part or claustrum insulare consists mainly of large cells somewhat similar to those found in the insular cortex. The ventral part — claustrum prepiriforme contains smaller cells. The border between the dorsal and ventral parts is in the bottom of the rhinal sulcus.
It is difficult to determine whether the insular and the prepiriform claustrum constitute a morphological and functional unity. These doubts are due to the fact that the prepiriform claustrum passes upwards not only in the insular claustrum but also in the deepest layers of the cortex. The prepiriform claustrum is called by some authors the endopiriform nucleus (13). The insular-claustrum is separated laterally along its entire length from the insular cortex by a more or less distinct capsula externa. The capsula externa adheres medially to the insular claustrum, distinctly separating it anteriorly from the putamen and posteriorly from the amygdaloid body. The anterior part of the insular claustrum appears on the frontal sections as a rather narrow band of cells stretching from dorso-lateral to ventro-medial. The middle part, the largest, is on the frontal sections triangular in shape. In the posterior part the insular claustrum is elongated dorso-ventrally and it narrows assuming the shape of a club.

The pretectal area also called the pretectal region comprises a transient area between the diencephalon and the mesencephalon. In mammals this area is not distinctly separated and there is a lack of uniformity with regard to nomenclature of its nuclei. Incomplete knowledge of the afferent and efferent connections of this area constitutes an additional difficulty in defining them accurately. The habenula and the posterior thalamic nuclei form the anterior border of the pretectal area. The posterior border is formed by the superior colliculus which possesses a layered structure. Laterally the pretectal area reaches to the nuclei of the posterior part of the thalamus, especially nucl. limitans. Medially it meets the posterior commissure and the periaqueductal grey. The ventral border is difficult to determine; it reaches the reticular formation of the mesencephalon.

In our studies the division of the pretectal area into respective nuclei is based with some exceptions, on the one proposed by Kanaseki and Sprague (26). The nomenclature of these authors is used by the majority of those working on this part of the cat brain. The pretectal area was divided into three parts: The antero-lateral part contains the anterior pretectal nuclei (nucleus compactus and reticularis). The postero-medial part comprises the medial pretectal nucleus, olivary pretectal nucleus, nucleus of the optic tract and suboptic nucleus. The ventral part contains nuclei of the posterior commissure.

Our nomenclature differs greatly from that used formerly by various investigators. Some authors (38, 53), in their reports did not mention the anterior pretectal nuclei, while others (23, 40, 45) used this term to designate the neighbouring structures.
MATERIAL AND METHODS

Sixty adult cats of both sexes (2.0–3.5 kg) were used. Surgery was performed in aseptic conditions, general anaesthesia with Nembutal injected intraperitonally in doses of 30 mg/kg body weight was used. The animals were placed in a stereotaxic apparatus (type SEZ 3 1975), remodelled to enable reaching the claustrum from the lateral side. Stereotaxic parameters were chosen on the basis of the Jasper and Ajmone-Marsan Atlas (24).

In all animals the skull was opened and the dura mater cut over the appropriate area of the brain. Next a micropipette was introduced stereotaxically and by this means a 30% solution of horseradish peroxidase (HRP, Sigma VI) was given in amounts of 0.20–0.50 μl in 20 to 60 min.

In 40 cats HRP was administered to the claustrum from the lateral side through the insular cortex and extreme capsule. In 25 animals the area of infusion was limited only to the insular claustrum and only those animals were analyzed in detail. Three cats received HRP to the claustrum from above, the micropipette passing through the gyrus suprasylvius medius. In 2 animals HRP was injected from above into the putamen. In 3 cats the HRP solution was injected to the insular cortex. The injection involved those areas through which the micropipette passed when HRP was injected into the claustrum from the lateral side. In 12 animals HRP was injected to various regions of the sensorimotor, visual and auditory cortex.

After surgery the animals received Penicilinum procainicicum, Polfa (100 000 units a day for 1–3 days according to the planned time of survival). The animals were sacrificed 24–72 h after surgery under general anesthesia with Nembutal injected intraperitoneally in doses of 70 mg/kg body weight. Perfusion was carried out according to the Rosene and Mesulam method (42).

The brain fixed in this manner was removed from the skull and kept up to 24 h in the perfusion fluid. The brain was cut on a frontal plane. Every 4th section was incubated for 30 min in a 0.05% solution of 3,3’ diaminobenzidine in 0.05 M buffer Tris (pH 7.4) at room temperature. The next incubation step was carried out at room temperature for 30 min in an incubation solution with the addition of H₂O₂ (0.3 ml H₂O₂ in 100 ml solution). Sections mounted on the slides were dehydrated and stained with cresyl violet.

The specimens were examined under a light microscope of 200×, 100×, 30×. HRP cells were indentified in various subcortical structures. The presence of HRP granules was studied also against a dark field. The labeled cells in various nuclei were counted on every fourth section.
RESULTS

Localization of HRP labeled cells following lateral injections to the claustrum

In 25 cats the HRP injection and diffusion area involved mainly the middle part of claustrum insulare (Fig. 1, 2). In each of these cases the greatest number of HRP labeled neurons was found in the pretectal area ipsilaterally to the injection. In none of the cats were there any HRP labeled cells in the contralateral pretectal area. Where the amount of peroxidase was smaller (within limits of 0.20–0.30 µl) there were about three times fewer HRP labeled cells than in those animals where injections were larger (from 0.35 to 0.50 µl).

Fig. 1. HRP injection into the claustrum from the lateral side. Frontal sections of the hemisphere through the middle part of the claustrum. Cresyl violet.
HRP labeled cells appeared in the pretectal area almost entirely in the anterior nuclei: compactus (NPAc) and reticularis (NPAr). They were mostly small and of diverse shapes. In the reticular nucleus HRP labeling appeared sporadically. In all the cats the distribution of labeled cells in these nuclei was similar. It seems that a more anterior or posterior injection-site within the middle part of the claustrum had no greater significance for the location and number of HRP labeled cells in the pretectal area.

Fig. 2. Scheme of localization of HRP injection into the claustrum. Frontal sections of the claustrum (A–I) in rostrocaudal order. Injection site is marked in black. Dots represent diffusion area.

The labeled neurons were much more numerous in the nucleus compactus (NPAc) than in the nucleus reticularis (NPAr). In the compact nucleus the greatest number of labeled cells was found in its middle
part, fewer in its posterior part and only single labeled cells were found chiefly in its anterior part (Fig. 3, 4, 5). The numerous labeled neurons were also found on the other side of the thin lamina separating pretectum from the thalamus, in the most medial part of the nucleus limitans.

In 3 cats labeled cells were found in other nuclei of the pretectal area. In cat C-6 there were 4 and in C-34 3 such cells in the posterior pretectal nucleus. In cat C-10 there were 8 labeled cells in the nucleus of the optic tract. In all these cases the labeled cells were localized in the anterior parts of the above nuclei.

Fig. 3. The distribution of HRP labeled cells in the pretectal area, following injections to the claustrum from the lateral side. Frontal sections.

Localization of HRP labeled cells following injection to the claustrum from above

In 3 animals where HRP was injected to the claustrum from above the infusion was restricted to the middle part of the insular claustrum (Fig. 6). Despite the different penetration track of the micropipette to the claustrum in both anterior pretectal nuclei a similar number of HRP labeled cells was visible as in the animals which received the same volume of enzyme to the claustrum from the lateral side. The distribu-
Fig. 4. Dark-field microphotographs of labeled cell from the middle part of the compact anterior pretectal nucleus, following HRP injection into the claustrum.

Fig. 5. Dark-field microphotographs of large triangular labeled cell from the middle part of the reticular anterior pretectal nucleus, following HRP injection into the claustrum.
tion of labeled cells was also similar, as their greatest number was found in the middle part of both anterior pretectal nuclei (Fig. 7). These labeled cells did not differ in shape and size from those observed in the previous group of animals.

![Fig. 6. Scheme of localization of HRP injection into the claustrum from dorsal side. Frontal sections of the claustrum (A–I) in rostrocaudal order. Injection site is marked in black. Dots represent diffusion area.](image)

**Localization of HRP labeled cells after injections to the putamen**

In 2 animals small injections of HRP were made to the putamen. The micropipette was directed from above as in the previous group. The enzyme was administered to the part of the putamen bordering on the middle part of the claustrum through the external capsule. In both animals the injection slightly involved the external and internal capsules. It is difficult to state with absolute certainty whether the diffusion slightly involved the claustrum or not. In the pretectal area after such injections there are a few labeled cells in both anterior pretectal nu-
clei. No labeling was found in the remaining nuclei of pretectal area. The cells were also located in the middle part of both anterior pretectal nuclei, their shape and size not differing from those previously described.

Fig. 7. The distribution of HRP labeled cells in the pretectal area, following injection to the claustrum from the dorsal side. Frontal sections.

Localization of HRP labeled cells after injections to the cortex

HP (0.5 μl) was injected to various parts of the cortex. In 5 animals the injection involved the visual and neighbouring cortex, in 4 — the sensorimotor, in 3 — the auditory and in 3 — the insular (Fig. 8, 9). In the majority of animals the infusion was restricted to the cortex only. However, in some cases HRP also reached the subcortical fibres. HRP labeled cells were not found in the pretectal area in any of the animals of this group.

DISCUSSION

The question requiring a more extensive discussion is how far the labeling of cells in the anterior pretectal nuclei may be taken as a proof of connections emerging from the above nuclei and terminating in the
Fig. 8. Cat's brain seen from the lateral side, with marked places of HRP injections to the cortex. C-58, 59, 60 auditory cortex, C-46, 47, 48 insular cortex.

Fig. 9. Cat's brain seen from the dorsal side with marked places of HRP injections to the cortex. C-54, 55, 56, 57 sensori-motor cortex. C-49, 50, 51, 52, 53 visual cortex.
claustrum. As is well-know HRP passes through the membranes of axon terminals and from there it is transported to the perikarya of the parent cells. Owing to this fact, by administering HRP to various nuclei of the central nervous system it is possible to study their afferent connections. HRP, however, penetrates the axons not only through the membranes of nerve terminals. This may also be a result of damage of the axons in the track of the micropipette or at the injection-site (10). It should also be taken into consideration that HRP diffuses from the injection-site to adjacent areas which may also affect the results obtained by this method.

In order to exclude the possibility of HRP getting to damaged axons in the path of the micropipette, two stereotactic approaches to the claustrum were used. One, lateral passing through the insular cortex and extreme capsule. The other, from above, through the cortex of the upper surface of the brain and white matter of the hemispheres. We observed that the localization of HRP labeled cells in the pretectal area was similar in both groups of animals. Therefore the labeling of HRP cells is not due to the damage of the fibres on the micropipette track from the surface of the hemispheres to the claustrum. One must also consider the possibility of HRP entering damaged axons passing through the claustrum on their way from the pretectal area to other structures. Although the site of HRP infusion was defined in all the animals, it is also necessary to consider the possibility of a slight diffusion from the claustrum to the putamen because of their proximity. This required HRP injections to the putamen and various areas of the cerebral cortex, as a control material.

The injections in the putamen revealed some cells in the anterior pretectal nuclei. One may presume that the presence of these cells might have been due to the existence of additional projections from the anterior pretectal nuclei to the putamen. The enzyme also could have entered the pretecto-claustral axons passing through the putamen. Neither can we exclude the fact that the HRP diffusion might have involved not only the putamen but also, to a slight degree, the adjacent part of the claustrum.

In these considerations the essential fact is that when the same amount of HRP was injected to the putamen as to the claustrum the number of labeled cells in the anterior pretectal nuclei was incomparably smaller. This suggests that HRP labeling in the anterior pretectal nuclei following claustral injections was not caused by the injections of pretecto-striatal connections.

HRP in various cortical areas did not cause the labeling of pretectal cells. It would thus seem that the projections from the pretectal area
do not terminate in the cortical areas involved in HRP injections. This does not entirely exclude the possibility that some axons arising in the pretectal area run through the claustrum to other areas of the cerebral cortex. There is little probability, however, of so large a number of axons running through the claustrum from the pretectal area to small, distant cortical areas. Therefore the data presented here speak strongly in favor of direct connections of anterior pretectal nuclei with the claustrum.

Another question to be answered is: can the projection from anterior pretectal nuclei to the claustrum be considered as the main or one of the main subcortical afferents of the claustrum? In all the animals receiving injections to the claustrum the number of HRP labeled cells in the anterior pretectal compact nucleus is very high. It is generally known, however, that not all neurons which send axons to the areas to which HRP was injected are revealed by this method (29). This depends on many factors, among others on the number of collateral and terminal branches (9). Therefore the lack of HRP labeled cells does not always signify the lack of connections. Thus we cannot claim with entire certainty that anterior pretectal nuclei are the source of the main subcortical projection to the claustrum, although they seem to send a large claustropetal projection.

As already mentioned, up to the present, data on subcortical connections of the claustrum are scanty. It was supposed that the claustrum receives projections from the thalamus (11, 22 and 34). Electrophysiological changes in the claustrum as a result of stimulation of the centrum medianum and other intralaminar nuclei appeared to support this assumption (49). There are data that the nerve fibres of some parts of the extrapyramidal system such as striatum and substantia nigra (1) terminate in the claustrum. Also from our unpublished data it appears that the thalamus and some nuclei of the extrapyramidal system may send connections to the claustrum. These projections, however, seem to be scanty in comparison to the afferents which the claustrum receives from the anterior pretectal nuclei.

The projections emerging from anterior pretectal nuclei may be divided into descending and ascending. The ascending projections for the most part approach the reticular thalamic nucleus, central lateral thalamic nucleus, and zona incerta (4, 5, 17 and 52). The descending projections chiefly approach the perirubral area, a part of the reticular formation related to the somato-motor system and to the medial part of the superior colliculus (4, 5, 51, 52). Other reported efferent connections of the pretectal area, running to the nuclei of the posterior commissure, inferior olivary nucleus and substantia grisea pontis (51, 52),
arise probably not in the anterior pretectal nuclei but in other parts of the pretectal area.

Efferent connections emerging from the anterior pretectal nuclei terminate for the most part in areas considered as subcortical sensory nuclei. The majority of these nuclei receive nerve fibers that convey somatosensory impulses. Hassler (20) supposes that central lateral nucleus of the thalamus, reticular thalamic nucleus and zona incerta belong to the sensory nuclei of the first integrative level of the sensory impulse.

The functional significance of anterior pretectal nuclei was until recently unknown, while the role of the claustrum in the central nervous system has not yet been explained satisfactorily. The majority of investigators relate the pretectal area to the optic system (5, 6, 18, 19, 26, 44). Their experiments show that the fibres from the retina terminate bilaterally within the pretectal area, mainly in the nucleus of the optic tract, in the pretectal olivary nucleus and partly in the posterior pretectal nucleus.

Magoun and Ranson (30) found a relationship between the pretectal area and the light reflex. The Argyll Robertson symptom is supposed to depend on injury of this area. Sprague (47, 48) found that following damage to the pretectal area there was no correct reaction to objects moving within the visual field. A large part of the pretectal area receives projections from the visual cortex (16, 27). It was shown that field 17 and 18 projects to the posterior pretectal nucleus and nucleus of the optic tract. The majority of observations supports the hypothesis that the nucleus of the optic tract and pretectal olivary nucleus possess both afferent and efferent connections with the subcortical areas related to vision such as ventral nucleus of the lateral geniculate body, pulvinar and accessory optic nuclei (3, 5, 6, 15, 50). Data pointing to a close relationship of the pretectal area and the visual system concern almost exclusively the nuclei of the dorso-caudal part of the pretectal area and nuclei of the posterior commissure. On the other hand, as appears from the literature, the anterior pretectal nuclei do not take part in conveying visual information.

According to Berkley and Mash (4) there is a large projection upon the anterior pretectal nuclei from the somatosensory cortex. Later investigations of Berkley (2) on cats and monkeys revealed a large projection from the nuclei of the posterior funiculi to the anterior pretectal nuclei. It seems that sensory nuclei of the trigeminal nerve also project to this area as does the lateral cervical nucleus and part of the fibres of the spinothalamic tract. All the above data suggest a close relation of the anterior pretectal nuclei to the somatosensory system.
The functional significance of the claustrum is unknown. There has been only one publication concerning behavioral studies following injury to the claustrum; Chorzążyna (8) showed the role of the claustrum in analysing auditory stimuli. Removal of the auditory cortex in the dog did not affect its ability to distinguish stimuli of different frequency, whereas removal of the claustrum caused irreversible changes in this respect. Electrophysiological studies on the claustrum (21, 46) do not give equivocal results. It was found that it is a polisensory part of the brain. Its neurons may respond to somatosensory, auditory as well as visual stimuli. The majority of claustral neurons, however, reacts to one type of stimuli, mainly somatosensory ones. Olson and Graybiel (37) located the somatosensory projection in the middle part of the claustrum.

It is presumed (21) that sensory impulses reach the claustrum by a roundabout path through specific nuclei of the thalamus and cerebral cortex (the so-called cortical loop). However, some physiological data suggest a direct path that conveys sensory input to the claustrum by-passing the cerebral cortex. These suppositions were not backed by any anatomical data.

The results presented here lead to the hypothesis that pretecto-claustral axons may constitute such a type of direct connections. Thus somatosensory information may be conveyed to the claustrum from the sensory nuclei of spinal and cranial nerves and from nucleus gracilis as well as nucleus cuneatus by means of only one mediating neuron localized in the anterior pretectal nuclei. It would therefore appear that there are two pathways conveying sensory impulses to the claustrum one, longer, passing through the thalamus and cortex, the other, shorter, running directly through the somatosensory part of the pretectal area. In the light of the above data it may be supposed that the claustrum under the influence of the inflowing sensory information may affect large areas of the cerebral cortex. This influence exerted by numerous claustro-cortical connections may involve the activity of cortical neurons as well as some behavioral processes (8).

LIST OF ABBREVIATIONS

Aa — Area amygdaloidea anterior
Ca — Commissura anterior
Ci — Claustrum insulare
Cp — Claustrum prepyriforme
CSC — Commissura colliculi superioris
Gp — Globus pallidus
HAB — Nuclei habenulae
NPAc — Nucleus pretectalis anterior compactus
NPar — Nucleus pretectalis anterior reticularis
NPC — Nuclei commissurae posterioris
NPM — Nucleus pretectalis medialis
NPO — Nucleus pretectalis olivaris
NPP — Nucleus pretectalis posterior
NPSO — Nucleus subopticus
NTO — Nucleus tractus optici
PC — Commissura posterior
Pt — Putamen
Py — Cortex prepyriformis
Rh — Sulcus rhinalis

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