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Neuromorphologie

EFFERENT PROJECTIONS OF THE CLAUSTRUM TO THE DORSAL AND VENTRAL STRIATUM, SUBSTANTIA NIGRA, VENTRAL TEGMENTAL AREA AND PARABRACHIAL NUCLEI. RETROGRADE TRACING STUDY IN THE RAT

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Abstract

The subcortical efferent projections of the claustrum in the rat were investigated by means of retrograde tracing using Fluoro-Gold as an effective retrograde fluorescent marker. The tracer was stereotaxically injected unilaterally in the dorsal ("extrapyramidal") striatum, in the nucleus accumbens (ventral, "limbic" striatum), in the substantia nigra, in the ventral tegmental area, and in the parabrachial nuclear complex in the dorsolateral pons. In all cases with successful infiltration of these structures, the ipsilateral claustrum contained retrogradely labelled neuronal perikarya. The projection towards the dorsal striatum arises from loosely distributed neurons, intermingled with claustrocortical cells, over the entire territory of the claustrum. On the other hand, the axons to the nucleus accumbens arise from neurons concentrated towards the medial border of the claustrum, close to the external medullary lamina. The projections to the extrapyramidal structures, e.g. dorsal striatum and substantia nigra arise only from the claustrum proper, whilst the tracts to structures, affiliated with the limbic system (nucleus accumbens, ventral tegmental area) are emitted also by the endopiriform nucleus (the ventral continuation of the claustrum). Unexpectedly strong connection from both the claustrum and endopiriform nucleus to the parabrachial nuclear complex is presently reported. The functional significance of this newly described projection remains to be

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elucidated but it is plausible to speculate that it might be concerned with the pain processing.

Key words: basal ganglia, dorsolateral pons, extrapyramidal system, Fluoro-Gold, limbic system, nucleus accumbens, polysensory integration

Introduction. The claustrum is a thin strip of gray matter that lies in the white matter between the lentiform nucleus and the insular cortex. It is separated from these structures by the external capsule medially and the extreme capsule laterally. The most significant efferent connections of the claustrum reach the entire neocortex and many allocortical areas ([1–3] and references therein). These connections are topographically organized, and are reciprocated by massive corticoclaustral tracts [4]. Whereas the connections of the claustrum with the cerebral cortex are firmly established, reports on the subcortical efferent projections of the claustrum are both scant and contradictory. Claustrum projections to several thalamic nuclei were reported in retrograde and anterograde tracing studies [5,6]. After stereotaxic destruction of the claustrum, DRUGA [7] followed degenerating axons to the putamen by the means of selective silver impregnation method of Nauta. ROSEGAY [8] believed that the posterior claustrum innervates the substantia nigra and adjacent tegmentum.

Following the retrograde tracing study of the afferent connections of the amygdala [9], we started the investigation of the efferent connections of the amygdala by both anterograde tracing [10] and retrograde tracing. While observing retrogradely labelled neurons in the amygdala following injection of the tracer in different nuclei, we noticed in several cases retrogradely labelled cells also in the claustrum. We found it reasonable to report these data, since some of them are largely neglected in the present literature, and the projection of the claustrum to the parabrachial nuclear complex is a completely original finding.

Material and methods. All procedures were carried out according to a standard protocol established by the Ethic Commissions at the Medical University of Sofia, and at the University of Rostock, Germany. Twenty male Wistar Albino rats weighing 220–260 g were used. The deeply anaesthetized rats were mounted in David Kopf stereotaxic apparatus in the flat skull position. Using a Hamilton syringe the animals received 0.25–0.5 µl 2% Fluoro-Gold (FG) in the dorsal striatum, nucleus accumbens (ventral striatum), lateral part of substantia nigra, ventral tegmental area – medial part of substantia nigra, and the parabrachial nuclear complex. The coordinates were obtained from the atlas of PAXINOS and WATSON [11]. After a survival time of four days, the animals were deeply anaesthetized and perfused transcardially with 100 ml phosphate buffered saline, followed by 500 ml 4% paraformaldehyde in phosphate buffer (pH 7.2), and finally with 100 ml of the same fixative to which 5 g sucrose were added. The brains were removed and stored in 20% sucrose in the same fixative for 2–30 days at 4 °C. Serial sections were cut at a thickness of 30 µm on a Reichert

Jung freezing microtome and stored in phosphate buffer overnight at 4 °C. The sections were mounted on chrome alum gelatin coated slides and allowed to dry overnight at room temperature. The sections were completely dehydrated in 100% ethanol, cleared in xylene and coverslipped with Entellan. The sections were observed in Nikon and Leitz Aristoplan fluorescent microscopes equipped with filter set with excitation length 350–395 nm. Photomicrographs of selected fields were taken with a digital camera (7.3 three Shot Colour, Visitron Systems, Diagnostic Instruments).

Results. In four experiments the FG injections were placed in the dorsal striatum. In one experiment there was a spillage of the fluorescent dye upon the overlying cortex and this case was excluded from examination due to the contamination of the mighty claustrocortical projection. Another experiment was partially successful since the injection focus infiltrated also the dorsal portion of the nucleus accumbens. In two experiments the injection focus was limited to the dorsal striatum and only the periphery of the injection halo reached ventrally the nucleus accumbens (Fig. 1a). Numerous retrogradely labelled neurons were seen in the ipsilateral dorsal claustrum (Fig. 1b-d). The fluorescing neurons were loosely distributed but were present in almost all claustral areas except from its large (prestriatal) rostral pole. Retrogradely labelled neurons were seen in the rostral claustrum (Fig. 1b), in the central portion of the claustrum (Fig. 1c), and approaching the caudal pole of the claustrum (Fig. 1d), where the number of labelled neurons gradually diminished.

In four experiments the FG injections were placed in the nucleus accumbens. Two cases with larger injection foci were excluded from systematic examination due to the involvement of the dorsal striatum, and in one of these cases the focus involved posteriorly the bed nucleus of the stria terminalis. In the remaining two cases the injection focus in nucleus accumbens was selective (Fig. 1e), and only the periphery of the injection halo reached the dorsal striatum and the septum (dorsally), and the olfactory tubercle (ventrally). Both cases with selective infiltration of nucleus accumbens displayed a characteristic pattern of labelling in the claustrum. The fluorescing neurons were concentrated in the medial portion of the dorsal claustrum, close to the external capsule. Ventrally the band of retrogradely labelled neurons imperceptibly continued in the endopiriform nucleus (the ventral claustrum, see the Discussion).

In four experiments the FG injections were placed in the transitional zone between the substantia nigra and the ventral tegmental area. In three cases, one of them illustrated in Fig. 1g, the injection focus involved both structures. In one case the injection focus was placed more dorsally and involved the dorsal portion of the ventral tegmental area. In the three cases with infiltration of the tracer in the ventral tegmental area and medial part of substantia nigra, also a very slight variation of the injection focus was followed by discrete differences in the retrograde labelling pattern. In the case with infiltration mainly of the

ventral tegmental area (Fig. 1g) the retrograde labelling was present both in the claustrum and in the endopiriform nucleus (Fig. 1h). In another case the focus involved mainly the medial part of the substantia nigra. Here, the number of labelled neurons in the claustrum was slightly larger, while in the endopiriform nucleus only few fluorescing neurons were present. In all three cases, however, mainly the larger claustral and endopiriform cells were retrogradely labelled. By the focus in the dorsal part of the ventral tegmental area occasional labelled neurons were seen in the claustrum, and somewhat larger number of cells appeared in the endopiriform nucleus. By medial injections no labelled neurons in the lateral striatum were seen (Fig. 1h).

In four experiments the FG injections were placed in the lateral half of the substantia nigra. In one case the focus was displaced dorsocaudally and involved the caudolateral mesencephalic tegmentum, leaving the caudolateral substantia nigra in the periphery of the injection halo. In three cases the centre of the injection focus was placed in the lateral part of substantia nigra, pars reticulata, and involved also the lateral part of the substantia nigra, pars compacta, and the substantia nigra, pars lateralis (Fig. 2a). In these three cases along the neuronal labelling in the dorsal claustrum, also a profuse retrograde labelling was present in the lateral part of the striatum, and the two populations were discriminated

Fig. 1. (a): A selective injection focus in the medial portion of the dorsal striatum. The necrotic tissue in the centre of the focus was removed during the histological preparation. Ventrally the periphery of the injection halo reaches the nucleus accumbens (Acb). cc – corpus callosum; LV – lateral ventricle; Spt – septum. Scale bar – 500 µm
(b)–(d): Retrogradely labelled fluorescing neurons in the claustrum, from rostral (b) to caudal (d). The labelled neurons are regularly distributed throughout the dorsoventral and mediolateral extents of the claustrum. In (d) also retrogradely labelled neurons in the deep layers of the cerebral cortex are seen. CC – cerebral cortex; ec – external capsule; Str – striatum. Scale bar – 100 µm
(e): A selective injection focus in the nucleus accumbens. The injection halo reaches but not infiltrates the septum and the striatum, and only slightly infiltrates the dorsomedial portion of the olfactory tubercle (OT). aco – anterior commissure; LV – lateral ventricle; Spt – septum. Scale bar – 500 µm
(f): The labelled neurons in the claustrum (Cl) occupy the medial zone of the nucleus, close to the external capsule. Ventrally the retrogradely labelled neurons are located in the endopiriform nucleus (En). A substantial number of retrogradely labelled neurons are scattered in the overlying cerebral cortex. Scale bar – 100 µm →
(g): Injection focus in the ventral tegmental area and the most medial part of substantia nigra. Due to intensive interneuronal connections, and to the involvement of ascending fibres by the injection, the injection halo appears unproportionally large. SNc – substantia nigra, pars compacta; VTA – ventral tegmental area. Scale bar – 500 µm
(h): Retrogradely labelled neurons in the claustrum. Part of them represent the largest claustral cells, and are strongly labelled. Occasional retrogradely labelled neurons in the deep cortical layers, most of them – weakly fluorescing. The striatum does not display labelled neurons, since its lateral portions do not project to the ventral tegmental area; compare with Fig. 2b. Scale bar – 300 µm

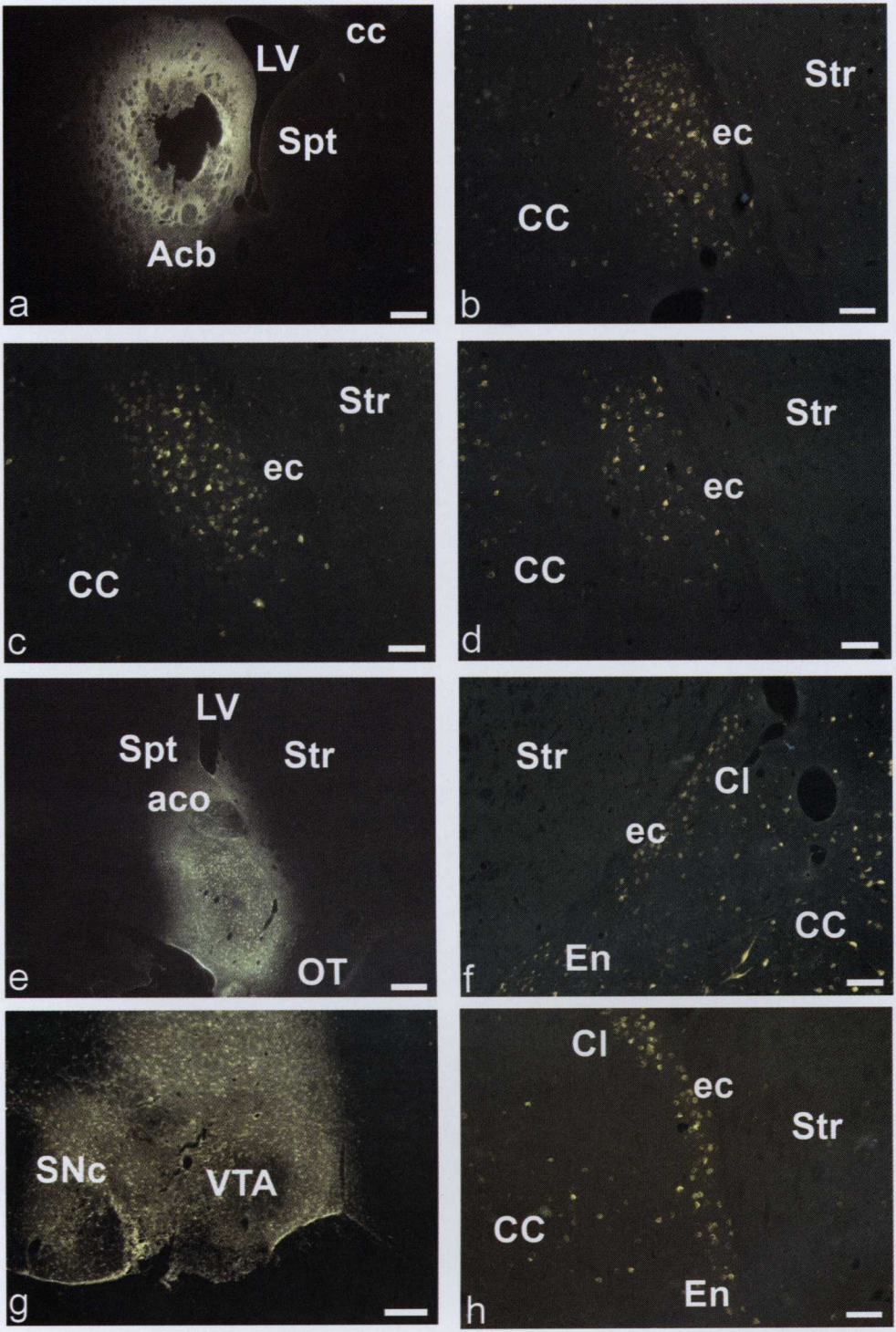
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Fig. 1

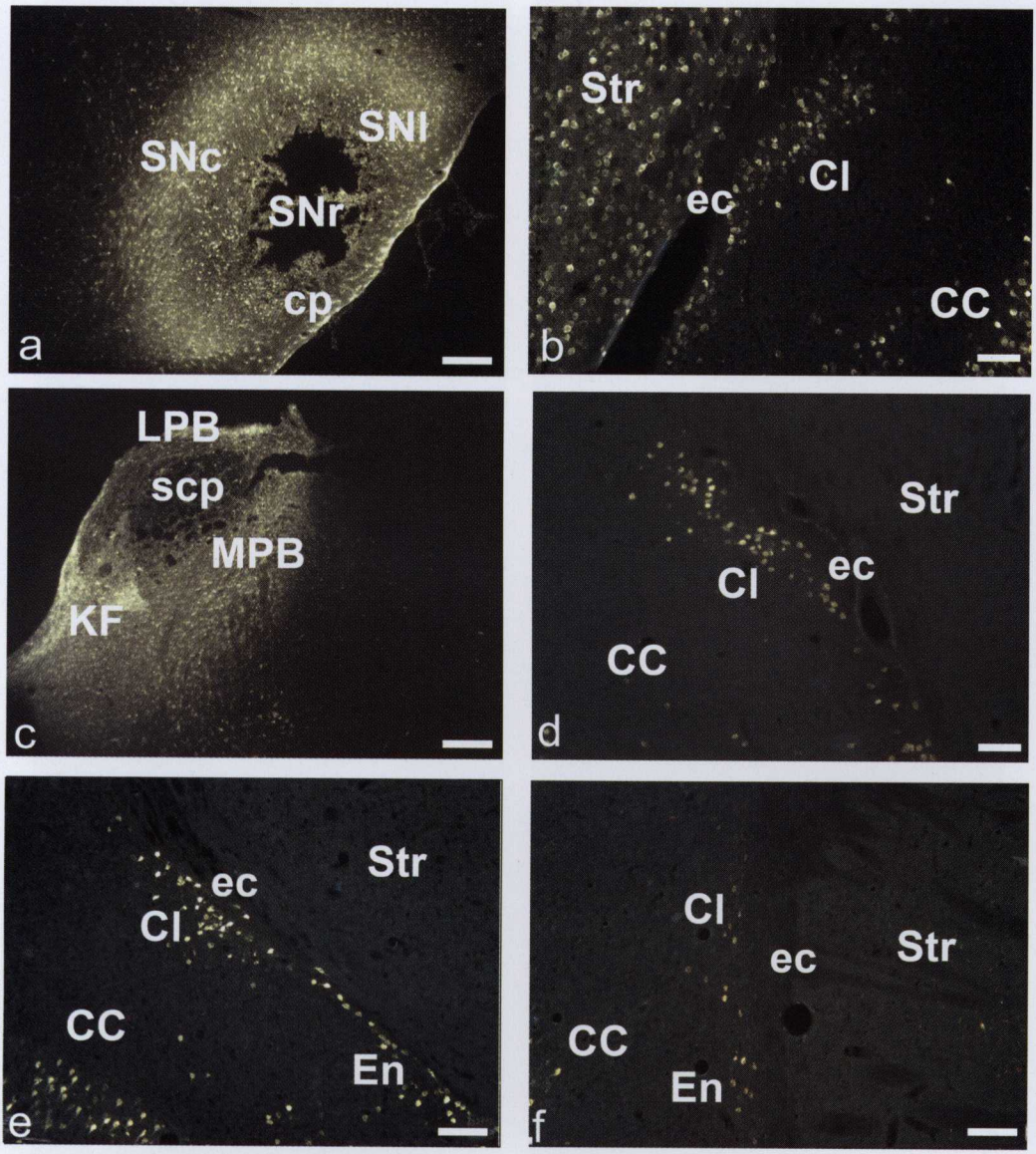
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Fig. 2

by the external capsule. Again, mainly the larger claustral neurons were found to project to the substantia nigra. Their number was larger in the dorsal claustral portion, and labelling was very scant in the endopiriform nucleus. In the dorsally displaced focus few scattered fluorescing neurons were present in both divisions of the claustrum.

FG injection in the parabrachial nuclear complex in the rostrodorsolateral pons was attempted in 4 rats, in two cases – successfully (Fig. 2c). The focus infiltrated the gray matter surrounding the superior cerebellar peduncle: the lateral and medial parabrachial nuclei, and the nucleus of Koelliker-Fuse. In one case the focus was located more dorsally and involved mainly the lateral parabrachial nucleus. In one case the focus was significantly displaced dorsally and involved the overlying cerebellar tissue. In both cases with successful infiltration of the parabrachial complex, a characteristic pattern of labelling in the ipsilateral claustrum was present (Fig. 2d-f). With exception of the rostral pole of the claustrum, the entire nucleus contained retrogradely labelled neurons. In the rostral claustrum (Fig. 2d) the fluorescing cells were concentrated mainly towards the external capsule. Moving caudally (Fig. 2e), in the central third of the claustrum, a clear accumulation of labelled neurons was encountered, and a strip of fluorescing neurons close to the external capsule connected the dorsal claustrum with the endopiriform nucleus. In the caudal part of the claustrum (Fig. 2f) only few retrogradely labelled neurons remained in the dorsal claustrum, whilst a larger number of fluorescing perikarya were present in the endopiriform nucleus. Notably, labelled neurons in the claustrum were present also in the case with a small injection involving the lateral parabrachial nucleus.

Discussion. In higher mammals, including man, the claustrum consists of two parts, the insular (or dorsal) claustrum, which underlies the insular cortex,

Fig. 2. (a): Injection focus in the lateral half of the substantia nigra. The centrally placed necrotic area is located in substantia nigra, pars reticulata (SNr). Dorsally the focus involves substantia nigra, pars lateralis (SNl), and medially – the lateral part of substantia nigra, pars compacta (SNc). cp – cerebral peduncle. Scale bar – 500 µm
(b): A substantial number of retrogradely labelled neurons in the claustrum. In the striatum there are numerous labelled strionigral neurons; compare with Fig. 1h. Scale bar – 100 µm
(c): A selective injection focus in the caudal half of the parabrachial nuclear complex, involving the medial parabrachial nucleus (MPB), lateral parabrachial nucleus (LPB) and
← the nucleus of Koelliker-Fuse (KF). The dark region in the centre of the focus is the superior cerebellar peduncle (scp). Scale bar – 300 µm
(d)–(f): Pattern of retrogradely labelled neurons in the claustrum and endopiriform nucleus following an injection of FG in the parabrachial nuclear complex. The claustrum contains a significant number of retrogradely labelled neurons in the rostral (d) and central (e) portions of the claustrum. The retrogradely labelled neurons are aggregated mainly in the medial portion of the nucleus, close to the external capsule. Few retrogradely labelled neurons in the caudal pole of the claustrum (f). Ventrally to the claustrum also retrogradely labelled neurons in the endopiriform nucleus are observed (e,f). Scale bar – 100 µm

close to the putamen, and the prepiriform (or piriform, also ventral or temporal) claustrum, located dorsolateral to the amygdaloid nuclear complex [4]. In lower mammals the ventral claustrum is usually nominated "endopiriform nucleus" and precise cytoarchitectonic studies divide it into a dorsal and ventral endopiriform nuclei [11]; for a recent review see [6].

The claustrum projects to the entire cerebral cortex [1–4], so that "this structure bears a unique and striking resemblance with the thalamus" [4,12]. There are discrete somatosensory, visual and auditory subdivisions in the claustrum interconnected with the corresponding sensory areas of the neocortex [2,4,12,13], and there are data pointing out that the claustrum is strongly involved in nociception [14].

The subcortical efferent projections of the claustrum are far less elucidated. Druga [7] was the first to describe a claustrostriatal tract in the cat but with few exceptions [5] this laborious Nauta study was overlooked. Recently ZHANG et al. [6] followed anterogradely labelled fibres from the rostral claustrum in the rat, and traced them mainly to the shell of the nucleus accumbens, "... in contrast to the relative absence of fibres in the core of the accumbens". Zhang et al. [6] mentioned no connections to the dorsal striatum. We established projections of the claustrum to both the dorsal ("extrapyramidal") striatum, and to the ventral striatum (the nucleus accumbens). The pattern of retrograde labelling is different. The neurons projecting to the dorsal striatum are somewhat loosely distributed but fill almost the entire territory of the claustrum, intermingled with the neurons that project to the cerebral cortex, as described in [2]. Only the most rostral claustral neurons were not found to project to the dorsal striatum that corresponds with the negative results of Zhang et al. [6]. The neurons projecting to the nucleus accumbens form a condensed strip in the medial part of the claustrum, close to the external capsule. Moreover, following injections of FG, labelled neurons appear also in the endopiriform nucleus, whilst this structure obviously does not innervate the dorsal striatum. Thus, probably the claustral projections to the striatal subdivisions arise from separate cell populations.

The first suggestion that the claustrum possesses subcortical connections was reported already in 1944 by Rosegay [8]. He transected the substantia nigra and adjacent tegmentum in cats and reported significant retrograde degeneration in the posterior claustrum, whilst the retrograde degeneration in corpus striatum was less pronounced. For nearly 30 years this study was often cited but more recent studies regarded it sceptically. First, the contemporary tract tracing studies clearly indicated that the strionigral neurons are absolutely refractory to retrograde degeneration changes due to intensive intrastriatal collateralization (broadly reviewed in [4]). Second, the first evidence that the claustrum projects heavily upon the cerebral cortex was provided exactly following the observation of retrograde changes in the claustrum following lesions of the cerebral cortex [15]. In all probability, Rosegay [8] encountered the topically distributed in the posterior

claustrum cortically projecting cells following a concomitant injury of claustrocortical axons. The claustronigral connection was reinvestigated only recently by Zhang et al. [6]. They traced anterogradely the axons of the anterior claustrum, and reported that a relatively light labelling was present in the medial areas of both the reticular and compact parts of substantia nigra. According to our experiments, the claustrum projects to the lateral and medial parts of substantia nigra, and to the ventral tegmental area. In both experimental series, mainly larger claustral neurons were labelled. Only the dorsal claustrum was found to project to the lateral portions of substantia nigra, whereas following injections in the medial substantia nigra and in the ventral tegmental area also labelled neurons in the endopiriform nucleus were present.

The interpretation of the functional significance of the claustrostriatal and claustronigral projections is difficult, since not only the morphological but also the physiological data on the involvement of the claustrum in the basal ganglia circuitry are scant. There is evidence that the claustrum is involved in the coordination of motor control [16], and that the claustrum is implicated in epileptogenesis [6,17]. Therefore, one might also expect monosynaptic connections between the claustrum and "classical" motor structures as striatum and substantia nigra.

As it is pointed out in the Introduction, the injection of the tracer in the parabrachial nuclear complex was attempted for the investigation of the descending efferent connections of the amygdala, and the presence of retrogradely labelled neurons in the claustrum was an unexpected finding. To the best of our knowledge, this is the first communication reporting a projection of the claustrum and endopiriform nucleus to the dorsolateral pons. Perhaps, some of the axons reaching the parabrachial nuclei represent collaterals of fibres to substantia nigra, periaqueductal gray and dorsal raphe nucleus [6] since some of the claustral neurons are only weakly fluorescing. However, there is also a substantial number of neurons both in the claustrum and in the endopiriform nucleus that are strongly fluorescing (see especially Fig. 2e), indicating that the parabrachial nuclei are the main target of these cells. Only tentative speculations might be presented on the functional significance of the presently described projection. The parabrachial nuclei represent a relatively small but complicated gray mass that are implicated in diverse functions: relay centre of gustatory and general visceral afferent information, regulation of respiratory, cardiovascular and gastrointestinal activity, etc. (see [9,18] and references therein). As it was mentioned above, the claustrum appears to be involved in nociception [14], and it seems probable that exactly the pain circuitry wires the claustrum with the parabrachial nuclei, since there is growing evidence that the parabrachial nuclear complex is greatly involved in pain processing [9,19,20]. In addition, the parabrachial nuclei coordinate different types of sensation, and the claustrum is reciprocally interconnected with practically all sensory cortical areas. Thus, one might speculate that the interconnections of the parabrachial nuclei with the claustrum represent a part of multineuronal chains concerned with polysensory integration.

In conclusion, along the profuse innervation of the cerebral cortex, the claustrum emits also diverse subcortical efferent connections. The interconnections of the claustrum with dorsal striatum and substantia nigra indicate the involvement of this structure in the motor networks of the basal ganglia, while the projections to the ventral tegmental area and nucleus accumbens suggest an influence to both significant mesolimbic dopaminergic structures. The functional significance of the projections to the parabrachial nuclear complex remains to be elucidated, but it is plausible to speculate that these connections might be concerned with pain processing.

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