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Proiezioni dal claustro alle regioni prefrontali e striatali coinvolte nel circuito prefrontale dei gangli della base

Claustral projection to prefrontal and striatal regions involved in the Prefrontal Cortex-Basal Ganglia Circuits

Relatore:
Chiar.mo Prof. LEONARDO FOGASSI

Correlatrice:
Chiar.ma Prof.ssa ELENA BORRA

Laureanda:

GIADA COLUCCIO

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ABSTRACT

The Claustrum (CLA), is a thin sheet of gray matter, located bilaterally between the putamen and the insula. The main input to the CLA comes from the cerebral cortex and its main output is back to the cortex. Recently, it has been demonstrated that the CLA projects also to the striatum. This study investigates whether regions of the CLA projecting to the prefrontal cortex project also to the head of the caudate and the rostral putamen, which are target of the prefrontal cortex and take part in the so called "prefrontal circuit" of the basal ganglia. The results of this study show a topography within the CLA, in which rostral/dorsal claustral regions projecting to the head of the caudate (more rostrally) and the rostral putamen (more caudally) largely overlap with those projecting to the orbitofrontal (more rostrally) and dorsolateral prefrontal (more caudally) areas. The prefrontal areas receive projections also from a more ventral/caudal claustral sector. These connections suggest that the CLA has a role in coordinating the activity of distant cortical regions and subcortical structures within the same functional network. According to functional studies in rodents, the CLA could control the prefrontal cortex activating inhibitory cortical circuits.

1. INTRODUCTION

The control of behavior and executive functions depends on several brain structures including the prefrontal cortex and the circuitry including the basal ganglia. This circuitry plays a crucial role in controlling actions, identifying, and achieving behavioral goals and in reinforcement-learning the activity of different cortical and subcortical regions. The reinforcement-learning is a process for which in each context, an action that has been associated with a reinforcement, positive or negative, is more likely to be chosen systematically in the future in the same or similar contexts. Executive functions are those 'high-order' cognitive processes that control purposeful behavior and the adaptive responses classically associated with the functions of the prefrontal cortex, also depending on its connection with the anterior cingulate cortex, the insula, the intraparietal sulcus, other regions of the posterior parietal cortex and the inferotemporal cortex. Furthermore, the prefrontal cortex and the basal ganglia together interact with parietal and posterior areas in voluntary movement control and action planning (Gerfen & Bolam, 2017, Làdavas & Berti, 2020).

1.1 PREFRONTAL CORTEX (PFC)

The prefrontal cortex (PFC) is composed by a set of neocortical areas critically involved in high order brain functions such as planning complex cognitive behaviors, personality expression, decision making, control of social conduct, attention, planning and sequencing of actions and emotional regulation. A pivotal role of this region concerns cognitive control and actions in accordance with one's goals as well as problem solving and fluid intelligence. PFC is also involved in “top-down” processes. i.e., when the behavior is guided by the intentions of the subject based on an internal goal (Làdavas & Berti, 2020).

An injury of the frontal lobe does not produce noticeable intellectual deficits, but rather clinical features defined as “frontal lobe syndrome”: the characteristics of this syndrome include reduced ability to sustain attention and impaired concentration, impulsiveness, poor judgment, inappropriate social behavior, inability to plan, organize and execute complex behavior (Krudop, W. A., & Pijnenburg, Y. A., 2015).

PFC can be distinguished from motor and premotor areas for the presence of a pronounced internal granular layer IV and its conspicuous reciprocal connections with the mid-dorsal (MD) nucleus of the thalamus (Wise, 2008). The PFC also receives afferents from all sensory associative areas and can therefore be qualified as a higher order heteromodal associative area, since integrate sensory data, motor feedback, and other information with memories (Petrides et al., 2012).

1.1.1 NEUROANATOMICAL SUBDIVISION OF PFC

The Prefrontal Cortex can be subdivided into three main regions: lateral (LPFC), medial (MPFC) and orbital (OPFC). Since OPFC and MPFC share different aspects related to connections and functions, they are commonly considered as constituents of a single complex, the orbital and medial prefrontal cortex (OMPFC). In the following sections LPFC and the OMPFC will be described separately.

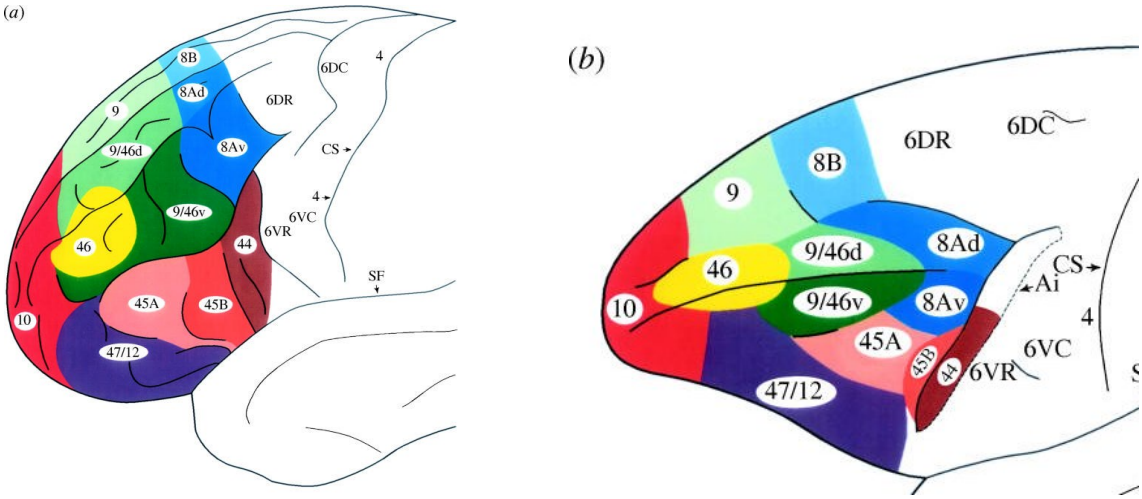

Fig.1 Cytoarchitectonic map of the lateral surface of the prefrontal cortex of (a) the human brain and (b) the macaque monkey brain by Petrides & Pandya (1994). Abbreviations: Ai, the inferior arcuate sulcus; CS, central sulcus; SF, Sylvian fissure.

1.1.2 LATERAL PREFRONTAL CORTEX (LPFC)

In non-human primates, the lateral prefrontal cortex (LPFC) is further separated into its dorsal and ventral sectors, and contains the Brodmann's areas 8, 9, 10, 45, 46, and 47. Phylogenetically, LPFC is more recent than other PFC regions and originates from motor regions such as the supplementary premotor and premotor area, and portions of the basal ganglia involved in motor behavior. LPFC also represents the highest level of sensorimotor integration, as with its connections it is responsible for the functional control and regulation of information processing. Sensory information is conveyed to the LPFC by conspicuous projections that originate from unimodal and polymodal sensory areas. The information is processed by the projections it sends and receives from occipital, temporal and parietal areas that process visual, auditory, and somatosensory information. Furthermore, LPFC plays a prominent role in planning and organizing goal-directed behaviors thanks to the reciprocal connections with premotor, anterior cingulate, and parietal areas. Studies on monkeys conducted by Rizzolatti et al. (1998) have shown the

presence in the agranular motor cortex of at least seven areas including the F1 area, which corresponds to the primary motor cortex. The other six areas, on the other hand, are subdivisions of the premotor cortex, the Brodmann area 6, later redefined as premotor. All areas have been named with the letter F, which means front, from F1 to F7. So, we have the area F3 and area F6 which are part of the mesial premotor cortex, the areas F2 and F7 which together make up the so-called dorsal premotor and, finally, F4 and F5 which are part of the ventral premotor.

LPFC exerts a control over motor behavior through a cascade of short projections, reaching the dorsal and ventral premotor areas (F2 – F5) and then indirectly the primary motor cortex (F1). Through top-down processes, LPFC processes "cognitive", goal-directed, aspects of motor control, such as sequence generation and motor learning (Làdavas & Berti, 2020).

An injury of LPFC leads to a “dysexecutive syndrome”, which includes reduction of intuition and judgment, poor planning and decision-making capacity, alteration of attention, working memory and temporal organization of recent events; it also includes difficulties in keeping online a representation of the stimulus, in its absence. Moreover, lesions mainly of the right LPFC impact on the control of behaviors in non-habitual conditions, affecting motivational aspects and social conduct. The dorsal-lateral prefrontal area is involved as it is also the seat of shifting and working memory skills, causing memory deficits in the temporal organization (Godefroy et al., 2010).

1.1.3 ORBITO-MEDIAL PREFRONTAL CORTEX (OMPFC)

The medial prefrontal cortex (MPFC) includes Brodmann's anterior limbic area 24, prelimbic area 32, and infralimbic area 25. BA areas 24 and 25 are located within the

boundaries of the rostral cingulate cortex and form part of the paralimbic girdle, while OFC covers the most ventral part of the PFC (Làdavas & Berti, 2020). Based on neuroanatomical and neurophysiological studies in non-human primates (reviewed in Ongür and Price,2000), there are two main circuits: one connecting orbital areas and one connecting medial areas. Part of the orbital areas are more connected with the medial areas and consequently are part of the medial network. Thus, orbital and medial networks are interconnected and as mentioned before, are often considered together as orbito-medial prefrontal cortex (OMPFC). Phylogenetically, together these areas have more ancient origins than the LPFC, evolving from limbic subcortical structures that mainly deal with visceral and emotional processing and for this reason it is classically considered as part of the limbic system (Làdavas & Berti, 2020). OFC in non-human primates consists of Brodmann area 11, 12 and 13 and mainly receives direct afferents from the parahippocampal regions and the amygdala, all regions considered critical for long-term memory, emotions, and processing of reinforcing signals (Barbas & De Olmos, 1990). The information OFC receives from visual, auditory, gustatory, and olfactory inputs of concerns the affective or emotional meaning of the stimuli. Emotions can be classified as positive or negative: positive emotions are evoked by positive gratifications or reinforcement, while negative emotions are evoked by negative punishments or reinforcements. Meanwhile, MPFC provides the major cortical output to visceromotor structures in the hypothalamus and brainstem. In particular, the OMPFC seems to be involved in decision-making processes based on 'value', impulse control, emotional processing, ethical and moral judgments. OFC is in fact closely linked to the activity of the reward dopaminergic circuit (reward system) (Làdavas & Berti, 2020). It is therefore not surprising that patients with OFC injuries often develop addictions to drugs and / or alcohol (Rudebeck, et al., 2008). It seems that the activity of medial part of OFC is related to the evaluation, learning and memorization of the value of gratification of positive

reinforcements, while the lateral part of the activity of the OFC is related to the evaluation of negative reinforcements that can determine modifications of the behavior. The OFC has a role in more complex behavioral changes that could be interpreted as inhibitory of behavior, this behavior arising in conjunction with activity in other brain structures, such as the anterior cingulate cortex (Gottfried et a., 2003).

Carmichael & Price (1995) showed that cytoarchitectonic sub-areas that form the medial and orbital prefrontal cortex are both included in a network of cortico-cortical connections. The orbital circuit includes a "sensory pole" involved in the analysis and integration of sensations relating to food. The medial circuit contains a "motor pole", which potentially exerts its influence on subcortical circuits that participate in the execution of motivated behaviors and includes also regions involved in autonomic and voluntary motor control. The orbital and medial network are strongly interconnected and, through these connections, the sensory information, examined for the relevant emotional and motivational aspects in the orbital network, is transferred to the medial network.

Patients with an injury of OFC often show the so-called “pseudopsychopathic syndrome” characterized by impulsiveness and inappropriate behaviors, up to the so-called “acquired sociopathy”. For example, they may show extreme slovenliness, hyperorality and hypersexuality, oppositional behavior, vulgarity, extreme aggression, are irritable, impulsive, and uninhibited, with disregard of social and moral principles. These patients therefore do not seem to consider the negative consequences of their actions, not only for themselves but also for others. According to some authors, patients with lesions to OFC and ACC and with impulse control deficits do not evaluate the consequences of their actions for others because they would lack the theory of mind and / or empathy and / or social intelligence (Antonucci, 2006).

Injuries to the OFC often induce dramatic changes in personality, as in the paradigmatic case of Phineas Gage, the most famous neuropsychological case and prototype of a patient with an OFC lesion. After an injury to his left PFC caused by a meter-long iron bar that lodged in Phineas's head, just below his left eye, including OFC, Gage showed no obvious cognitive or physical deficits, he was apparently normal, but he was no longer himself: he became rude, irreverent, blasphemous, disrespectful to people, intolerant of rules and obstinate. His case is emblematic because is the first scientific evidence indicating that damage to the frontal lobes might alter personality, emotions, and social interaction (Teles, 2020).

On the other hand, patients with MPFC damage usually show the so-called abulic frontal syndrome, which is characterized by the presence of apathy, indifference, affective flattening and, above all, by loss of initiative and cognitive flexibility, easy distractibility, and evident deficits to tasks that measure executive functions (M Das, J., & Saadabadi, A., 2022).

1.2 STRIATUM

Classically, the striatum has been considered part of the motor system, especially for its involvement in voluntary movements and particularly because functional damage to it causes severe motor pathologies. Now it is known that it also participates in cognitive functions, such as decision making, and reward-guided and habitual behavior (Burton et al., 2015). In addition, an injury of the caudate nucleus causes deficit in cognition, as revealed by delayed alternation exercises and shifting (Yeterian & Pandya, 1991). Neuroimaging studies showed that the striatum is involved in procedural processes of learning and memory (Packard & Knowlton, 2002).

Impairment of basal ganglia circuits in cortical motor areas leads to motor symptoms, whereas damage to subcortical components of circuits in cortical non-motor areas results in higher-order deficits. For example, Parkinson's disease begins with pathological changes that primarily occur in the sensorimotor part of the striatum (Kish et al.,1988). In contrast, Huntington's disease begins with pathological changes primarily in the associative parts of the striatum as the anterior caudate (Vonsattel, et al. 1985).

In primates, the striatum can be subdivided in a ventral and a dorsal part. The ventral striatum involves the nucleus accumbens, a ventromedial band of the caudate nucleus and part of the ventral putamen; the dorsal striatum includes the caudate nucleus and the putamen, which are separated by the internal capsule. The caudate nucleus is an elongated and arched cellular structure, consisting of a rostral part called the head of the caudate nucleus, a short intermediate body, and a long tail, while the putamen is a rounded structure located medially to the insula. Structurally, the caudate nucleus and the putamen are identical, have a homogeneous structure and contain numerous medium-sized neurons. The predominant cell-type of the striatum (75%) are medium-sized projection neurons with spines, called medium spiny neurons (MSN). In addition to MSN, there is

a consistent number of interneurons, all of which are spiny-free. These include large cholinergic cells and several types of small, medium-sized GABAergic elements. The projection cells of the striatum are considered inhibitory and use GABA as their primary neurotransmitter. The main input to the striatum consists of excitatory, glutamatergic fibers that originate from the cerebral cortex and from the intralaminar nuclei of the thalamus. Furthermore, the striatum is a target of dopaminergic projections from the substantia nigra and serotoninergic projections from the dorsal nucleus of the raphe (Haber, 2016).

1.2.1 CONNECTIONS AND CIRCUITS

The striatum is the main input station of the basal ganglia for the cortico-basal ganglia-thalamo-cortical loop. Several studies have established that the entire neocortex projects to both the caudate nucleus and the putamen and that the termination fields of projections originating from circumscribed cortical regions are organized in longitudinally arranged bands (Goldman & Nauta, 1977; McGeorge & Faull, 1989).

MSNs are traditionally divided into two groups, one with D1 dopamine receptors and the other with D2 dopamine receptors. D1 MSNs project to the basal ganglia output structures, i.e., the internal segment of the globus pallidus (GPi) and substantia nigra pars reticulata (SNr), forming the so called “direct pathway”, whereas D2 MSNs project to the external segment of the globus pallidus (GPe), at the origin of the “indirect pathway”. In fact, a widely used model of basal ganglia functioning relies on the D1/D2 direct/indirect pathway organization. In this model, the "direct" striatal projection system provides direct inhibitory input to the basal ganglia output neurons in the GPi and SNr, while the "indirect" striatal projection neurons provide inhibitory input to the GPe and the STN. The indirect pathway begins with D2-MSN suppressing the (GPe), which tonically

inhibits the subthalamic nucleus (STN). The STN receives input from the neocortex and provides excitatory projections to the GABAergic output neurons of the basal ganglia. The activity of the direct pathway results into an excitation of the thalamocortical projection neurons whereas the activity of the indirect pathway results into an inhibition, thus these two pathways modulate the cortical activity and, in the frontal cortex, inhibit movement execution (Gerfen, et al., 1990; Haber, 2016).

The thalamic nuclei receiving the pallidal projections are the ventral anterior and ventral lateral nuclei, which in turn project their efferent fibers to the motor, premotor, and supplementary motor areas, and the mediodorsal nucleus, which in turn projects to the prefrontal cortex, including the frontal eye field (FEF).

The major source of input to the striatum are projections from the ipsilateral cortical areas (Alexander et al., 1986), but the projections from motor and prefrontal areas of the contralateral hemisphere can be in some cases comparable to those of the ipsilateral one (Borra et al 2021). DeLong and colleagues (1984) have suggested that there are two distinct circuits which operate through the basal ganglia. One is a "motor" circuit that involves mainly the putamen, receives inputs from the sensorimotor cortex and converges on given premotor cortical areas. The other is a "complex" circuit that involves the caudate nucleus, receives afferents from integrative areas and whose projections ultimately return to specific regions of the prefrontal cortex. Other studies have proposed five functionally distinct circuits in which the basal ganglia would participate: motor, premotor, oculomotor, dorsolateral prefrontal, and limbic circuits (Alexander et al., 1986; 1990).

The motor circuit originates from the primary motor cortex, M1 (Brodmann’s area 4), which projects to the lateral putamen. This part of the putamen projects to the posterolateral part of the GP, which is connected to the anterior part of the ventral lateral

nucleus of the thalamus (VLa). This thalamic nucleus in turns projects back to M1. This cortico-basal ganglia-thalamo-cortical motor circuit is involved in controlling voluntary body movements (Alexander et al,1986; 1990).

The premotor circuit originates from Brodmann's area 6, which includes the dorsolateral premotor area (dlPM), the supplementary motor area (SMA) and the pre-supplementary motor area (PreSMA), placed on the medial surface of the hemisphere. PM and SMA are mainly related to the generation of the sequence of movements and motor learning (Alexander et al.,1986). Neuroanatomical studies on the monkey (Künzle H.,1975; Takada, 1998) have shown that the direct projections from the motor and premotor cortex to the striatum are organized according to a somatotopic scheme, in which the representations of the hindlimb and forelimb are located dorsally and ventrally, respectively.

The oculomotor circuit originates from the frontal eye field (FEF), which corresponds to Brodmann's area 8, and from the supplementary eye field (SEF), located in the rostral part of dorsal area 6. The corticostriatal projections originating from the FEF and the SEF converge on a striatal oculomotor area, which is in the central and lateral part of the head and body of the caudate nucleus. Efferences from the oculomotor striatum are directed to the substantia nigra pars reticulata (SNr) that, through the ventral anterior (VA) and mediodorsal (MD) nuclei of the thalamus, projects back to the FEF and the SEF. The nigro-thalamic fibers emit collaterals that descend to brainstem oculomotor structures. The cortico-basal ganglia oculomotor circuit is involved in the control of saccadic eye movements in complex behavioral contexts (Alexander et al.,1986).

The dorsolateral prefrontal circuit originates from Brodmann’s areas 9 and 46. The striatal territory receiving projections from these cortical areas includes a large part of the head of the caudate nucleus and also involves the caudate body and tail. These regions of the

striatum project to the dorsomedial portion of the GPi and to the rostral portion of the SNr, which, through the VA and MD nuclei of the thalamus, project to the dorsolateral prefrontal cortex. This circuit is involved in controlling cognitive functions, such as spatial and temporal planning of future behaviors, learning and working memory (Alexander et al.,1986).

The limbic circuit originates from areas of the OMPFC. The infralimbic areas project to nucleus accumbens, the prelimbic area projects to the nucleus accumbens and the caudate nucleus, the agranular insula projects to the ventral putamen and the later orbital area projects to the central part of the dorsal striatum. The ventral striatum projects to the ventral pallidus, i.e., the rostral and ventral extension of the GP. The GP projects to the medial and central parts of the MD nucleus of the thalamus that projects back to the prefrontal cortex (Alexander et al., 1986).

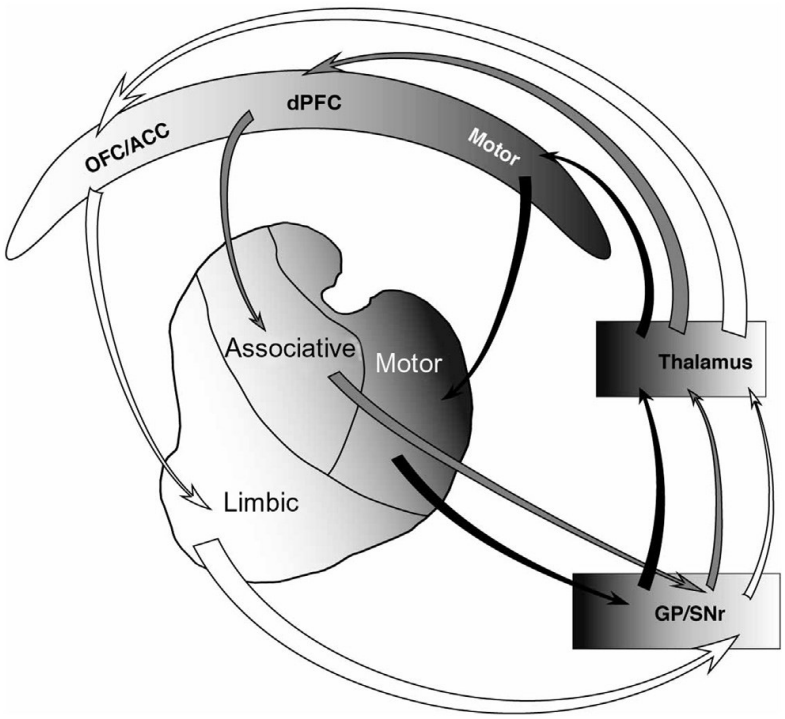

Fig.2 Schematic illustrating parallel circuits in corticobasal ganglia-cortical pathways. Corresponding shading in cortical, striatal, and downstream areas demonstrates topographic projections: white, limbic circuit; light gray, associative circuit; dark gray, motor control circuit. ACC, anterior cingulate cortex; dPFC, dorsal prefrontal cortex; GP, globus pallidus; OFC, orbital prefrontal cortex; SNr, substantia nigra pars reticulata. (Gerfen C. R. and Bolam J. P., 2017)

Although these cortico-basal ganglia- thalamo-cortical circuits are quite separated from each other, they originate from cortical regions that, in several cases, are interconnected (Herrero et al., 2002; Afifi, 2003; Bostan & Strick, 2018).

1.3 CLAUSTRUM

The claustrum (CLA) is a thin sheet of gray matter, located within the white matter of the cerebral hemispheres, bilaterally, placed between the putamen and the insula and extending, rostrocaudally, near the striatum. Its shape varies from species to species but appears to increase in size in proportion to the increase in neocortical volume. In primates it is thin and elongated dorsally, while ventrally it is thicker in almost its entire anterior–posterior extent. It has been investigated for several years but its precise function is already unknown.

The ontogenesis of the CLA still subject of discussions in the literature. According to Brodmann (1909), it originates from the sixth layer of the insular cortex, and also Bayer et al. (1991, 1993) suggested a cortical origin of the claustrum. However, according to Källén (1951) and Holmgren (1985), it should originate from the pallium. Recent evidence based on gene expression, indicate that the claustrum is different from both the cortical and striatal neurons (Watakabe et al., 2014)

The claustrum is clearly visible only in mammals, but it is still debated whether it is present also in monotremes (Butler et al. 2002, Ashwell et al. 2004, Baizer et al. 2014, Suarez et al. 2018). This observation would support the hypothesis of the involvement of the CLA in higher-order cognitive processes. The complexity of studying CLA lies in the obstacles caused by its tortuous shape, location, and closeness to other brain structures. In the past decades, based on new genetic and viral technologies, it was possible to discover new information on both its anatomical connectivity and physiological properties in rodents, that begins to reveal some aspects of its function (Jackson, 2020).

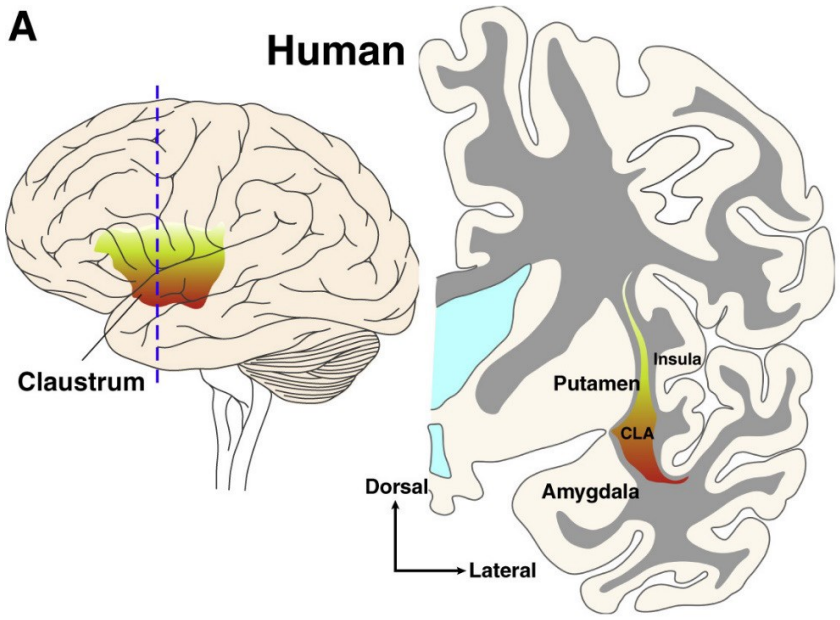

Fig. 3. Lateral vision of the extension of the CLA in the human brain in a coronal section (Smith et al., 2020).

1.3.1 ANATOMY AND PHYSIOLOGY

In the CLA, mainly two types of cells can be distinguished: cells of medium/large size, star-shaped or fusiform, with large spiny dendrites, whose axon leaves the body after giving off local collaterals (Golgi's type 1 cells) and small, granular, and spine-free cells with a locally arborizing axon who does not leave the claustrum, so they are categorized as interneuron. The dendritic spines are specialized to receive synaptic contact almost entirely from cells of the visual cortex (LeVay & Sherk, 1981; Crick & Koch, 2005).

Most neurons in the CLA are glutamatergic, excitatory projection neurons, while a small percentage of projecting neurons express nitric oxide synthase (NOS) (Kowiański et al., 2001) and thus should be inhibitory. Furthermore, neurons containing somatostatin and neuropeptide Y were found in the CLA. In addition to the communication between excitatory neurons, in the CLA there is also a synchronized inhibition of excitatory neurons by fast-spiking inhibitory, parvalbumin (PV)-positive, interneurons. Cortical inputs activate PV-positive neurons resulting in inhibitory postsynaptic potentials in

excitatory cells (Kim et al., 2016), which are synchronized precisely through this local inhibition.

Based on the differences in the morphology and in the relationships with the cortex, the claustrum can be divided into a compact dorsal part, and a more diffuse ventral or temporal part. The portion located above the level of the rhinal fissure, medial to the insular cortex, has been called the dorsal or insular claustrum, while ventral claustrum is medial to the piriform cortex, and was designated ventral or piriform claustrum and also endopyriform nucleus (Johnson & Fenske, 2014).

1.3.2 CLAUSTRAL CONNECTIONS

Kievit and Kuypers (1975) were the first to report the existence of direct projections from the anterior half of the claustrum to the frontal lobe cortex in the monkey.

Neuroanatomical studies in the macaque (Künzle H., 1975, Künzle & Akert, 1977, Mesulam et at., 1982, Minciacchi et al., 1991, Gamberini et al. 2017, 2020) have shown that numerous neocortical areas project to and receive from specific regions of the claustrum. The topographical organization of the cortical connections of the CLA in rodents revealed that most cortico-claustral inputs comes from the frontal lobe including structures like PFC, OFC, prelimbic, cingulate, and secondary motor cortex (Smith & Alloway 2010, 2014; Smith et al. 2012; Wang et al. 2017; Zingg et al. 2018). Moreover, a review of Mathur (2014) reports a matching in regional projections from the rostralmost cortical areas in the rostral part of the CLA and from the caudalmost cortical areas in the caudal part of CLA, suggesting the existence of modality-specific modules. These connections between CLA and the cortex are mainly reciprocal and ipsilateral, but some claustral neurons also project to the contralateral cortex (Crick e Koch, 2005; LeVay &

Sherk, 1981, Olson & Graybiel, 1980, Smith & Alloway 2010). In addition, the CLA is known to receive input from many subcortical limbic structures as the MD, the basolateral amygdala (BLA) and the hippocampus. Connections to the latter appear to be unidirectional, with CLA neurons receiving input from the ventral hippocampus (Zingg et al., 2018). The connections with the thalamus appear to be unidirectional, with the MD thalamus sending projections to the CLA, while the connections with the amygdala are bidirectional. Taken together, these rodent data about the subcortical limbic input of the CLA and its main PFC output suggest a role for the CLA as a limbic motor interface, enabling information about emotional valence and spatial navigation to guide action (Jackson, 2020).

Lévesque and Parent (1998) reconstructed axonal trajectories of neurons located in prelimbic cortex injected with biotin dextran or biocytin in rats, demonstrating the existence of cortical axons projecting to both the striatum and claustrum ipsilaterally. Furthermore, they found subpopulations of axons projecting to the striatum homolaterally and to the claustrum contralaterally and, in addition, the terminal area of the cortico-striatal-and-claustral fibers was located at the rostral and mid-dorsoventral levels of the CLA.

Clasca et al. (1992) showed, in the cat, that claustral cells projecting to the various cortical areas are topographically organized, as neurons projecting to the frontal areas are located in the anterior half of the claustrum, thus providing a rostro-caudal organization.

The projections of the claustrum to M1, PM, and PFC in macaques have been studied by Tanné-Gariépy et al. (2002), based on neural tracer injections. The labeled neurons after injections in all these areas/regions were distributed along the entire antero-posterior extent of the CLA. The cells projecting to all motor areas occupied the dorsal and intermediate parts of the CLA along the dorsoventral axis. Furthermore, visual zone of

the CLA has been identified in the ventro-posterior claustrum (Lysakowski et al., 1988; Mizuno et al., 1981), which is divisible into two regions, the posterior one that sends the output to areas V1, V2, V4, MT, MST and FST, and the anterior one that sends the output to the higher stages of the visual pathway, as the inferotemporal and intraparietal areas (Baizer et al., 1993). Furthermore, a large dorsal area of the CLA is connected to the somatosensory cortex in addition to M1 (Tanné-Gariépy et al., 2002; Minciacchi et al. 1991). Finally, the subpopulations of neurons that project to area 8 (FEF) and area 46 are located differently in the central and ventral part of the CLA, and also the projections from the CLA to the multiple sub-areas of the motor cortex and to area 46 originate from largely overlapping territories (Leichnetz and Goldberg, 1988).

Following striatal injections, Luppino et al. (2022) showed that CLA projects to the striatum in areas related to the hand of the motor and pre-commissural putamen.

Altogether, data collected in macaques clearly show that the major input to the CLA is from the cerebral cortex, and the major output of the CLA is to cerebral cortex and that the CLA is divided into multiple sensory and motor parts.

1.3.3 CORTICAL INHIBITION

Functional studies showed that the CLA can both inhibit and excite cortical activity (Cortimiglia et al., 1991, Salerno et al., 1984). Studies on cats showed that CLA neurons target mainly cortical layer II, III and VI (LeVay & Sherk 1981a, b, c, d; LeVay 1986; da Costa et al 2010). In mice, activation of CLA neurons projecting to mPFC through a viral approach using a Cre-dependent channelrhodopsin-2 (ChR2) virus in conjunction with a highly efficient retrograde AAV expressing Cre (Boyden et al., 2005; Tervo et al., 2016) caused the inhibition of neural firing in the mPFC (Jackson et al., 2018). Further evidence

of the inhibitory effect of CLA neurons on the cortex was provided also by two other recent studies, one on the auditory cortex (Atlan et al 2018) and one on the orbitofrontal cortex (Narikiyo et al., 2020), suggesting that the CLA plays a similar role for different cortical regions. Sternson & Roth (2014) reported that a chemogenetic suppression of PV-positive cortical interneurons does not lead to a total block of claustral-mediated cortical inhibition, while the suppression of interneurons containing neuropeptide Y (NPY) does. A key role for feedforward inhibition in corticoclaustral and claustrocortical communication in different species is also shown by the study of Narikiyo et al. (2020) and Norimoto et al. (2020).

1.3.4 HYPOTHESES OF CLAUSTRAL FUNCTION

Several hypotheses have been proposed for the function of the CLA. A recent hypothesis suggests its involvement in the regulatory mechanisms of sleep, as in rodents the CLA is active during REM sleep and increases its activity during slow-wave sleep compared to wakefulness (Narikiyo et al. 2020). Other studies in rodents have suggested a role of the CLA in regulating attention to sensory stimuli by inhibiting elements of distraction. Indeed, it has been shown that the CLA takes part in the salience network, which processes the emotional relevance of the stimuli, suggesting a role in directing attention to relevant sensory stimuli (Jackson, 2020).

Crick and Kock (2005) suggested a role for the CLA in consciousness, because its connections with cortical areas processing different sensory signals could be involved in creating a unitary perception of an event. Smythies et al. (2012) suggested that when a sensory information not matching the brain expectations comes from the thalamus, a mismatch signal is sent to the CLA and then from the CLA to several different cortical areas.

These hypotheses on the CLA function originate from the physiological information collected in rodent, mainly in studies on sensory processing in anesthetized animals. The role of the CLA in motor or cognitive functions is less studied but considering the abundant connectivity of the CLA with the frontal cortex, absolutely it deserves to be studied.

In conclusion, despite the studies carried out to date, the exact function of the claustrum is still unknown.

2. PURPOSE OF RESEARCH

The CLA has very widespread cortical and subcortical connectivity, which could provide it with the neural substrate for coordinating large-scale cortical and subcortical networks. Therefore, we analyzed the connectivity of the CLA with different cortical areas and striatal sectors to see whether the territories of the CLA that project to the PFC correspond to the territories that project to the caudate and the putamen that are involved in networks with the PFC.

For this purpose, we analyzed the distribution of the labelling in the CLA following injections into the caudate head and body and the anterior putamen and compared these distributions with those observed after injections in the OFC and DLPFC. To allow the comparison of the different cases, three-dimensional reconstructions of the CLA were obtained, and a merge procedure was carried out to make them comparable.

The work described in this thesis was carried out in the Neuroanatomy laboratory of the Department of Medicine and Surgery of the University of Parma, under the supervision of Prof. Giuseppe Luppino and Prof. Elena Borra.

3. MATHERIAL AND METHODS

3.1 SUBJECT

The experiments were carried out in 3 male Macaca mulatta (Cases 75, 76, and 77), in which retrograde neural tracers were injected in the putamen and the caudate (Table 1). Animal handling as well as surgical and experimental procedures complied with the European law on the humane care and use of laboratory animals (directives 86/609/EEC, 2003/65/CE, and 2010/63/EU) and Italian laws in force regarding the care and use of laboratory animals (D.L. 116/92 and 26/2014) and were periodically approved by the Veterinarian Animal Care and Use Committee of the University of Parma and authorized by the Italian Ministry of Health.

3.2 SURGERY

Under general anesthesia (In Case 75: induction with Ketamine 10 mg/kg, i.m. followed by intubation, isoflurane 1.5–2%; in case 76 and 77 induction with Ketamine 15-20 mg\kg i.v. and Medetomidine 0,05 mg\kg i.v.) and aseptic conditions, each animal was placed in a stereotaxic apparatus and an incision was made in the scalp. The skull was trephined to remove the bone and the dura was opened to expose the area of interest. The tracers were slowly injected under pressure by means of a stainless-steel needle attached via a tube to a Hamilton syringe (Hamilton Company, Reno NV). The needle reached the injection target region inside a "guiding tube" to prevent the tracer from leaking into the surrounding areas. Then, a time of ten minutes was waited with the needle inserted into the injection site to allow the tracer to diffuse and prevent it from leaking. After tracer injections, the dural flap was sutured, the bone was replaced with artificial dura, repositioned, and the superficial tissues sutured according to the anatomical planes. Injections were made in both the left and right hemisphere.

During surgery, hydration was maintained with saline (circa 10 ml/h i.v.), and heart rate, blood pressure, respiratory depth, and body temperature were continuously monitored. Upon recovery from anesthesia, the animals were returned to their home cages and closely observed. Dexamethasone and prophylactic broad-spectrum antibiotics were administered pre- and postoperatively, and analgesics were adimistered post-operatively.

3.3 INJECTION SITE SELECTION

Before the injection of neural tracers, the brain was scanned using MRI. The obtained images were used to calculate the stereotaxic coordinates in order to identify the best trajectory to choose for needle insertion, to reach the various sites of interest.

3.4 NEURONAL TRACERS AND HISTOLOGICAL PROCEDURES

Once the sites to be injected were chosen, the following tracers were injected: Diamidino Yellow (DY, 2% in 0.2M phosphate buffer at pH 7.4, EMS-POLYLOY GmbH, Gross-Umstadt, Germany), Fast Blue (FB 3% in distilled water, EMS-POLYLOY GmbH), the B subunit of the cholera toxin conjugated with Alexa 488 (CTB green, CTBg, 1% in PBS, Invitrogen-Molecular Probes, Eugene, OR, USA), dextran conjugated with lucifer yellow (Lucifer Yellow Dextran, LYD, 10,000 MW, 10% in 0.1 M Invitrogen-Molecular Probes phosphate buffer) and Wheat Germ Agglutinin, peroxidase-conjugated (WGA-HRP, 4% in distilled water, SIGMA, St Louis, MO, USA).

After the survival period necessary for axonal transport of the injected tracers (28 days for LYD, 14 days for FB, DY and CTBg and 2 days for WGA), each animal was first treated with atropine and then was deeply anesthetized with ketamine hydrochloride, sacrificed with a lethal injection i.v. of thiopental sodium (60 mg / kg) and then perfused through the left cardiac ventricle with, in sequence, buffered solutions of saline, 3.5%

paraformaldehyde and finally 5% glycerol. Each brain was then blocked coronally on a stereotaxic apparatus, removed from the skull, photographed, and placed in 10% buffered glycerol for 3 days and 20% buffered glycerol for 4 days, subsequently it was frozen by immersing it in pentane at -80 ° C. In Case 75, the right inferotemporal cortex was removed for other experimental purposes. Finally, each brain was cut frozen into coronal sections of 60-µm (case 75) or 50-µm (Case 76 and 77) thickness. In all the cases, sections spaced 300 µm apart were collected in six series to be used for different histological processing. In each series, slices are sampled every 300 µm, that is one section in each repeating series of 5 in Case 75 and one in series of 6 in Case 76 and 77. A series of sections was mounted, dried and quickly covered with coverslips for analysis under the fluorescence microscope. A second series was stained with the Nissl method (0.1% thionine in 0.1M acetate buffer pH 3.7).

In Cases 76 and 77, three other series were processed to display, respectively, WGA, CTBg, and LYD tracers for observation in brightfield. First of all, the sections were methanolized (methanol:hydrogen peroxide 3%, 4:1), with the aim of inactivating the endogenous peroxidases, and then they were incubated with a solution of primary antibody: i) anti-WGA (1: 2000, Vector Laboratories, Burlingame, CA, USA) in PBS 0.3% Triton and 5% normal rabbit serum (NRS) overnight at room temperature; ii) anti-Alexa 488 (1: 15000, Life Technology Eugene, OR, USA) in PBS 0.5% Triton and 5% normal goat serum (NGS) for two nights at 4 ° C; iii) anti-LYD (1: 3000) in TP 0.1M 0.3% Triton and 5% normal goat serum (NGS) for four nights at 4 ° C. Subsequently the sections were washed in PBS and incubated for one hour at room temperature with the biotinylated (1: 100), secondary antibody rabbit anti-goat for WGA and goat anti-rabbit for CTB and LYD, in PBS Triton 0.5 %, 5% NGS. After washing the PBS sections, the labeling was visualized with the Vectastain ABC kit (Vector Laboratories, Burlingame, CA, USA) using 3,3'-diaminobenzindine (DAB, Sigma, St. Louise, MO) as chromogen.

The reaction produced was intensified with cobalt chloride and nickel ammonium sulphate, and a black reaction was then obtained.

3.5 DATA ANALYSIS

In the present study, were analyzed the cells marked with retrograde WGA neuronal tracer in cases 76 and 77 and marked with retrograde DY neuronal tracer in case 75.

The analysis of labeled cells was carried out with a Nikon Eclipse 80i microscope for observations at medium and high magnification and for the acquisition of photos.

3.6 DATA COLLECTION

The material previously processed and mounted on a series of slides was digitized through a *custom-made software* for our laboratory (Brain Tracer, PACEM), to analyze the distribution of retrograde labeling in the claustrum ipsilateral to the injection, carrying out the analysis on sections 600 μm apart from each other. In each section, the border of the cortical surface, the border between gray and white matter and the border of the claustrum and the position of the labeled cells in the claustrum and surrounding white matter were traced. Using this software, the coordinates of the points located at the various edges, or the position of the cells were acquired, separately within a Cartesian reference system, using linear transducers, mounted on the X and Y axes of the translating stage connected to the microscope. The coordinates acquired for each section were then transformed into two-dimensional images by means of another software, also *custom-made* (Brainside, PACEM). Subsequently, the sections were transported to another *custom-made* 3D reconstruction software (Brain) to have a 3D representation of the claustrum and its labeling in the volumetric space of the structure. The sections have been aligned to obtain a faithful reconstruction of the anatomy of the CLA.

Case	Species	Age (years)	Weight (kg)	Brain Side	Tracer	Amount (µl)	Location
C75	M. mulatta	6	3.5	R	DY	0.25	Caudate body
				R	FB	0.7	OFC
				R	CTBg	1	Motor putamen
				L	LYD	1	Caudate head
C76	M. mulatta	9	15	R	WGA	0.1	Caudate head
				L	DY	0.4	Motor Putamen
				L	CTBg	1	Motor Putamen
C77	M. mulatta	9	15.35	R	WGA	0.2	Anterior Putamen
				L	DY	0.4	Motor Putamen
				L	CTBg	1	Motor Putamen

Table 1: Animals used, location of injections, type and amount of injected tracer

4. RESULTS

For the purposes of this study, three injections of tracers localized in the anterior putamen and in the head and in the body of the caudate were analyzed. The results obtained in these cases were then compared with those obtained after injections in different prefrontal areas (13/11, 46d, and 9), which are sources of projections to the caudate and the rostral putamen. Finally, one further case was used to identify the claustral territory projecting to the inferotemporal cortex, which is also source of projections to the rostral striatum.

4.1 LOCALIZATION OF CORTICAL RETROGRADE LABELING AFTER INJECTIONS IN THE STRIATUM.

All the injection sites used in this study were completely restricted to the target dorsal striatum (caudate or putamen).

In Case 76r WGA the injection site was in the caudate head 7mm rostral to the anterior commissure (AC). The labeled cells were located predominantly in the prefrontal cortex, mostly involving areas 9/8B and the rostral cingulate cortex, including area 24/32 and the cingulate gyrus. Relatively robust labeling was also observed in the temporal cortex, the superior temporal polysensory area (STP) and the medial temporal cortex, while a weaker labeling was observed in rostral premotor, insular, and caudal cingulate cortex. This labeling distribution pattern is in line with the location of the injection site in a region of the caudate involved in the prefrontal circuit.

In Case 75r DY, the injection site was located in the caudate body at the level of the AC, a caudate region functionally distinct form the caudate head. Indeed, the distribution of the labeling involved mostly dorsal and medial premotor areas, predominantly areas F3, F2, and F6 and to a lesser extent area F7. In area F3, the labeling mostly involved its rostral half. Less dense labeling was located in the ventral premotor cortex, motor cingulate, inferior parietal (AIP, PFG, PG) and caudal superior parietal (PEc, PEci, PGm,

V6A) areas. This labeling distribution suggests that the injection site involved a striatal sector related to visuo- and somatomotor control of arm movements.

One tracer injection involved the anterior putamen. The injection site in Case 77r WGA was located relatively ventrally in the pre-commissural putamen. The densest labeling was in the premotor cortex, especially area F5, frontal operculum, and rostral cingulate cortex, especially area 24c/d. Less dense labeling involved prefrontal, parietal, and insular cortex and, more weakly, temporal and caudal cingulate cortex. The distribution of the labeling involving the ventral premotor area F5, frontal operculum, rostral F3, ventrolateral prefrontal sectors including area 12 and ventral area 46, and inferior parietal and opercular parietal sectors suggests that the injection site involved a striatal sector related to hand and mouth motor control.

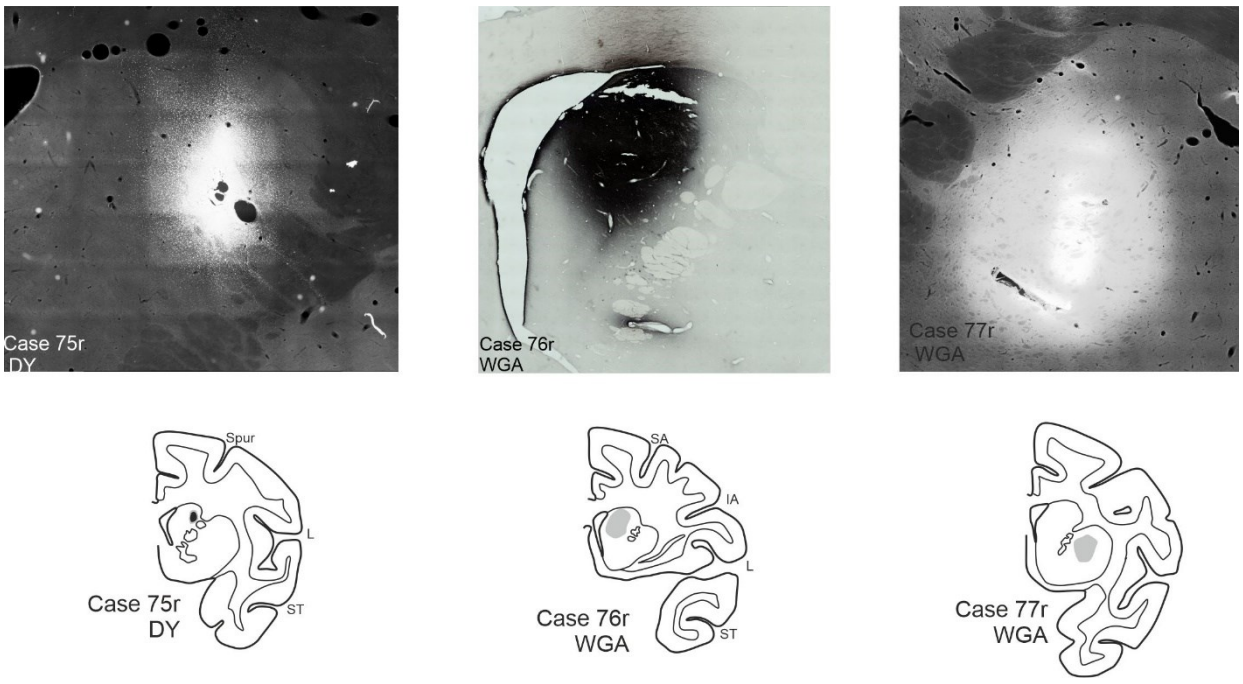

Fig. 4. Injection sites in the striatum of cases 75r DY, 76r WGA and 77r WGA.

4.2 DESCRIPTION OF RETROGRADE LABELING IN THE CLAUSTRUM AFTER INJECTIONS IN THE STRIATUM

Following injection in the head of the caudate in case 76r WGA, labeling was observed already from the most rostral slices and is mainly localized in the dorsal half of the CLA where it extends in the ventral direction, in front of the AC (Figure 5). Therefore, the labeled neurons are mainly localized in its dorsal and rostral part, mainly involving the region located rostrally to the AC, while in the central part of the CLA the labeling is sparse and in the caudal part is completely absent.

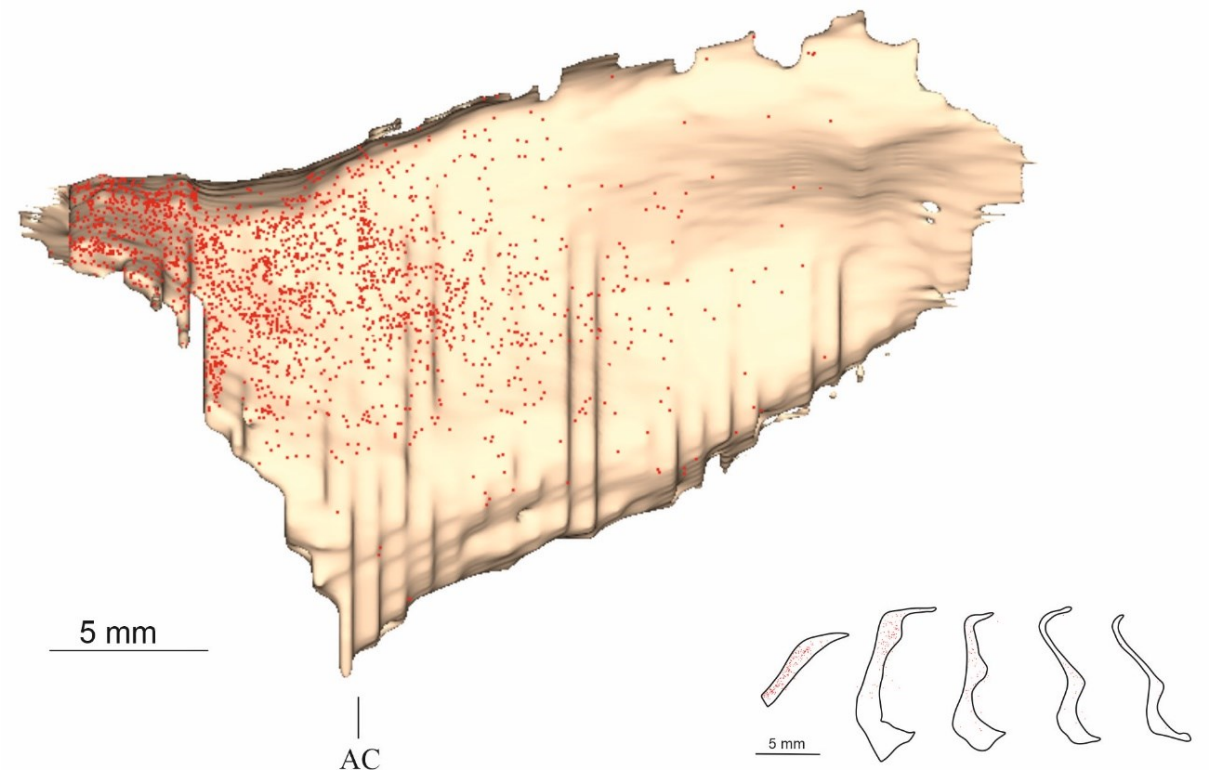

Fig. 5. 3D reconstruction and selected coronal section drawings of the CLA of Case 76r WGA.

After the injection into the body of the caudate in Case 75r DY, the labeling was located more posteriorly with respect to case 76r, avoiding the rostralmost part of the CLA, and involved an oblique rostro-caudal band running from the dorsal to the ventral part of the CLA (Figure 6.).

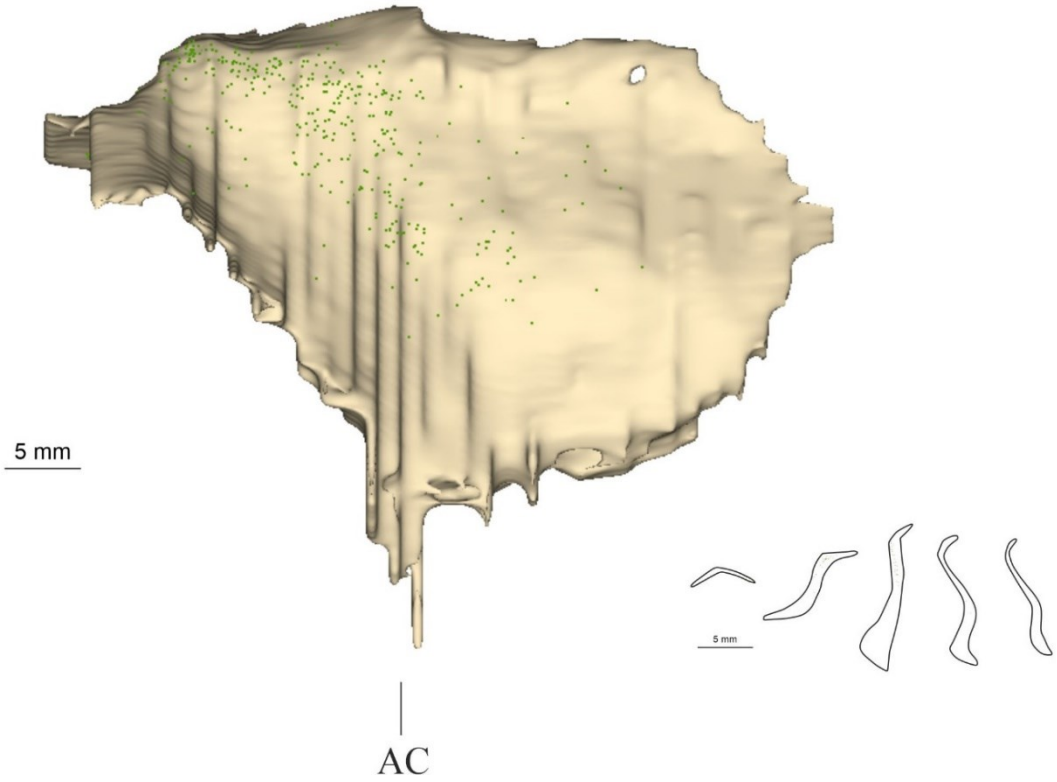

Fig.6. 3D reconstruction and selected coronal section drawings of the CLA of Case 75r DY.

The labeling observed after the injection in the anterior putamen in Case 77r WGA (Figure 7) was quite similar to that of Case 76r WGA. Indeed, the labeling was mainly located rostrally and dorsally, but avoided part of the rostral territory and extended more caudally with respect to that of Case 76r WGA.

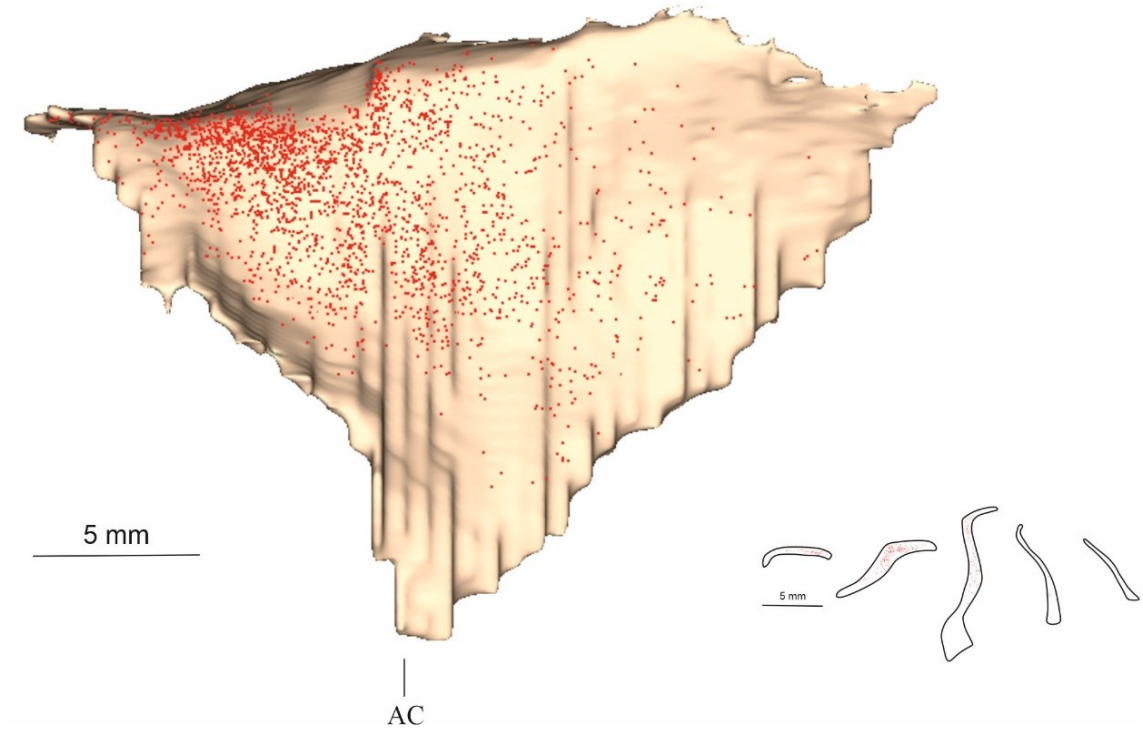

Fig. 7. 3D reconstruction and selected coronal section drawings of the CLA of case 77r WGA.

4.3 DISTRIBUTION OF RETROGRADE LABELING IN THE CLAUSTRUM AFTER INJECTION IN DIFFERENT PREFRONTAL AREAS

The distribution of the labeling in the CLA after injections in the rostral striatum was compared with that obtained after different prefrontal injections in areas that project to the rostral striatum (caudate head and rostral putamen). We therefore compared with a case of injections in dorsolateral area 46d (Case 60l FB, DY, CTBg, CYBr), area 9 (Case 58l LYD), and in orbitofrontal areas 11/13 (C75r FB).

In Case 60l, after FB, DY, and CTB-Alexa594 injections in area 46d at different rostrocaudal levels, an oblique band of labeled cells was located rostro-caudally from a dorsal to a ventral part of the CLA. The claustral region interested by the labeling is rather overlapping, especially rostrally, with the region labeled following the injection in the anterior putamen and in the head of the caudate. On the contrary, the labeling involved also a caudo-ventral part of the CLA that was not labeled after the rostral striatal injections.

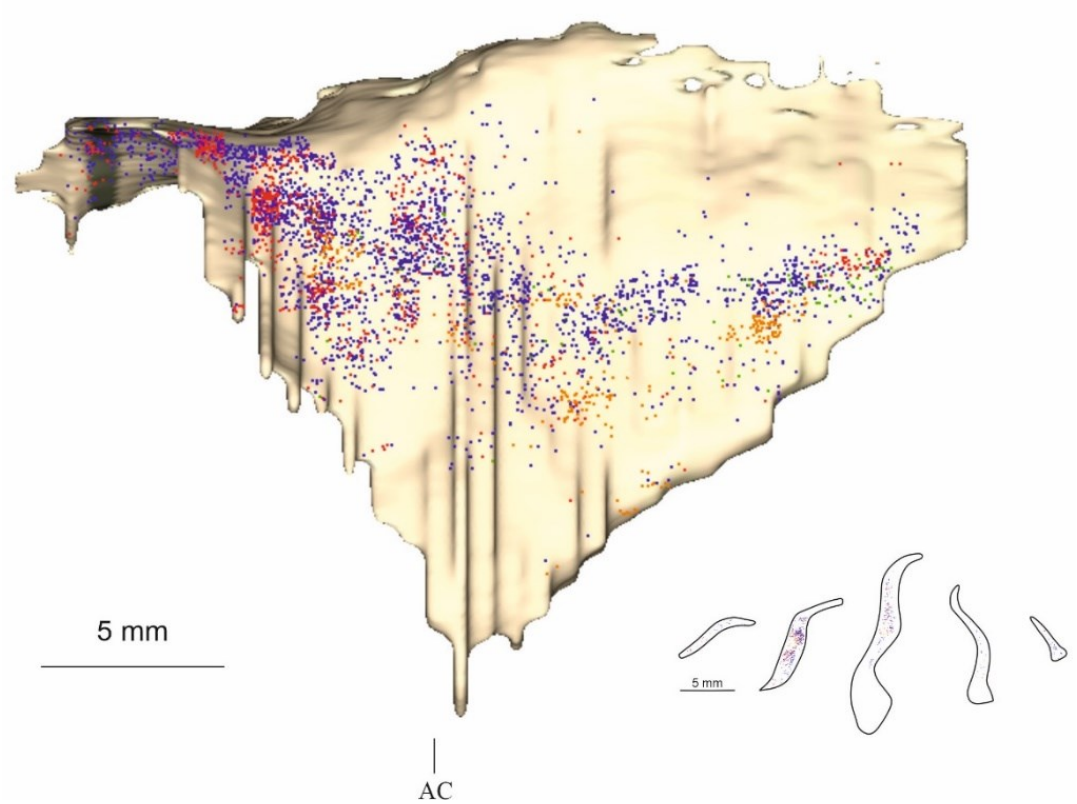

Fig.7 3D reconstruction and selected coronal section drawings of the CLA of case 60l: Fb in blue, DY in green, CTBg in orange, CTBr in red.

In Case 58l, after LYD injection in area 9, the retrograde labeling was very dense in the rostral part of the CLA and extended along the rostro-caudal direction occupying a dorso-ventral band, which seems to largely overlap to that of Case 60l but reaching also the ventralmost part of the CLA. The labeling in the ventral CLA was located mainly laterally.

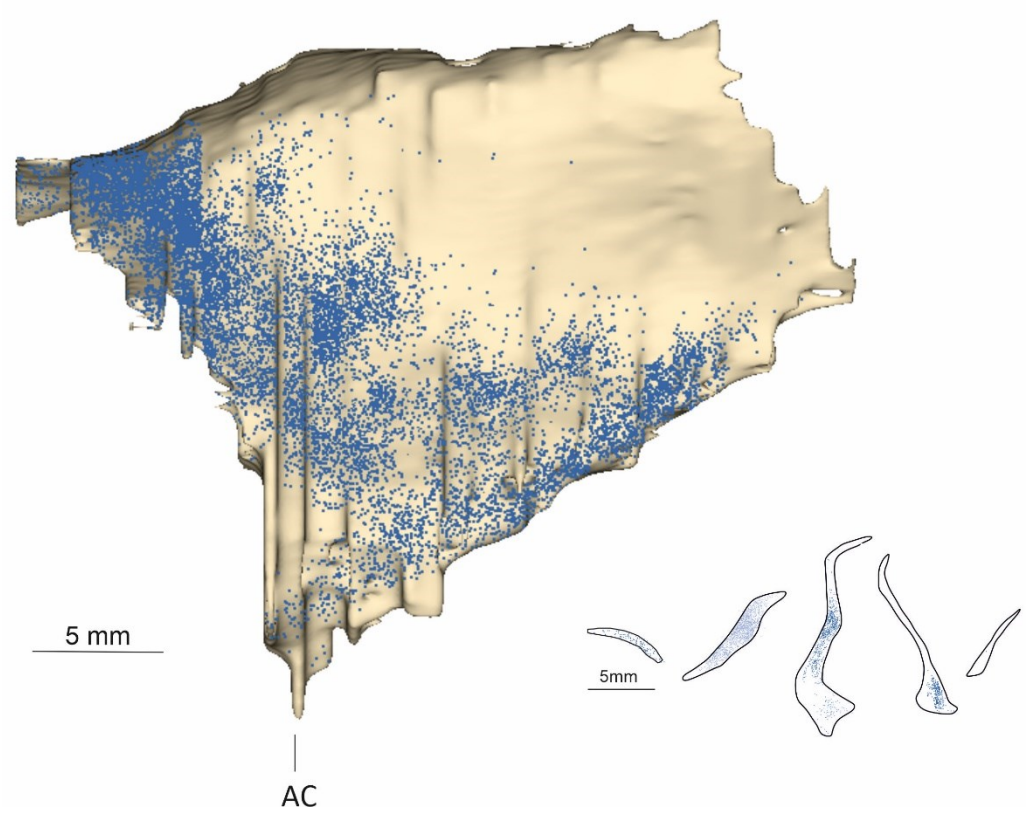

Fig.8. 3D reconstruction and selected coronal section drawings of the CLA of case 58l LYD

As can be seen in figure 9, the labeling observed after the injection in the OFC (case 75r FB) resulted in labeling located in an anterior band running at the lateralmost rim of the CLA, up to about the level of the AC. This labeling distribution is overlapping to part of the labeling observed after injections in area 9.

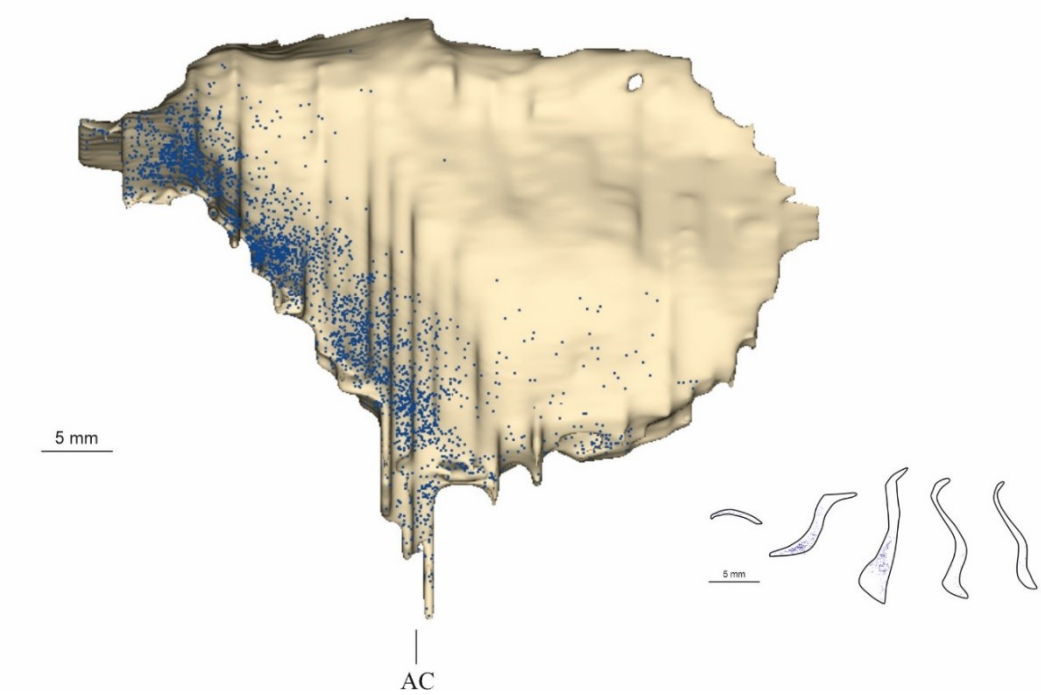

Fig.9. 3D reconstruction and selected coronal section drawings of the CLA of case 75 FB.

4.4 COMPARISON OF CASES

All cases of injection in cortical and subcortical regions involved in the prefrontal cortico-striatal circuit result in labeling located mainly in the rostral and dorsal half of the CLA. Figure 10 shows a composite view in which the cells projecting to the caudate and the putamen and the various prefrontal areas are shown merged on a template of a claustral 3D reconstruction. The claustral territory projecting to the caudate head and the anterior putamen is located in the rostrodorsal half of the CLA and the caudate head receive from a claustral sector extending more rostrally than that projecting to the rostral putamen. Caudal to the level of the AC the labeling becomes sparser. The cells projecting to areas 9, 46d, and OFC, are located in the same rostral and dorsal claustral region, but also in the ventral part rostrally and caudally with respect to the AC.

Accordingly, there is a rostro-dorsal sector of the CLA that projects to the rostral striatum and to dorsolateral and orbitofrontal prefrontal areas, which in turn project to the rostral striatum.

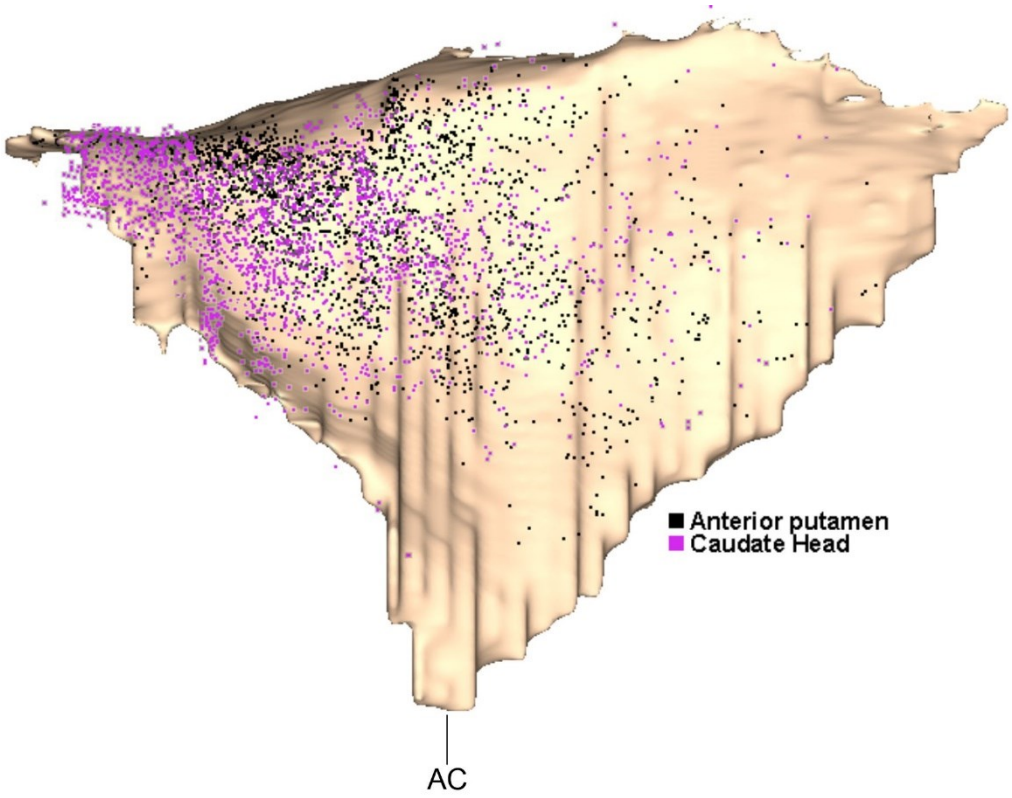

Fig. 10a

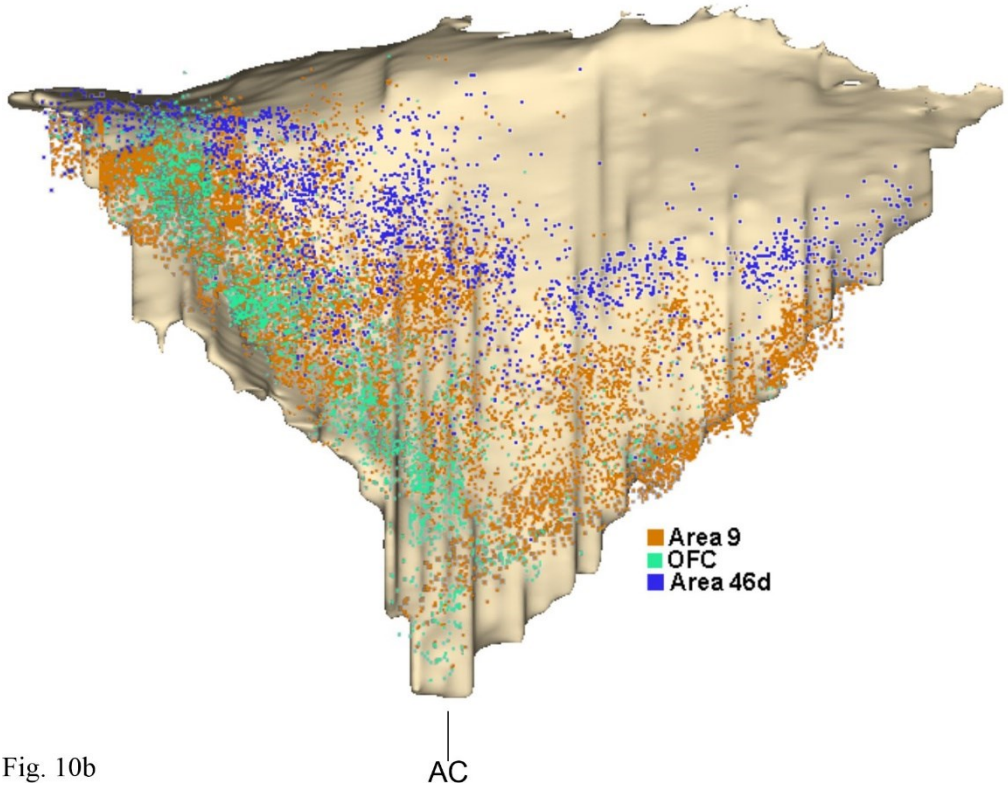

Fig. 10b

Fig.10a\b. Overlap of the labeling in the CLA after injections in different striatal (a) and cortical (b) regions.

5. DISCUSSION

In the present study, we observed a large overlap of the claustral territories projecting to the prefrontal cortex and the rostral striatum. Specifically, the rostralmost part of the CLA projects to the dlPFC (areas 46d and 9), OFC (area 11/13), and the caudate head, whereas a slightly more central part projects also to the anterior putamen. The claustral territory projecting to the prefrontal areas extends also more ventrally and caudally with respect to the one projecting to the anterior striatum.

5.1 COMPARISON WITH PREVIOUS STUDIES

Previous studies on the connectivity of the CLA in the macaque have analyzed the distribution of the labeling after injections in cortical areas belonging to the same lobe, such as prefrontal (Reser et al., 2014; Pearson et al. 1982), frontal (Tanne-Gariepy et al., 2002), parietal (Gamberini et al., 2017; 2021) and temporal (Gattass & Gross, 1981) area. Specifically, Reser et al. (2014), following injections in areas 9 and 10, found labeled neurons concentrated in a band running along the ventral portion of the CLA, such as the projection to area 12. After injections in areas 46 (Tanne-Gariepy et al., 2002) and 9 (Pearson et al. 1982), labeled cells were located along the rostral-caudal axis of the CLA. Projections to area 12 overlapped with those of area 9, but more ventrally and in the caudalmost portion of the CLA.

Based on these and other data, Gattass proposed different hypotheses on the organization of the claustral projections (Gattass et al., 2014).

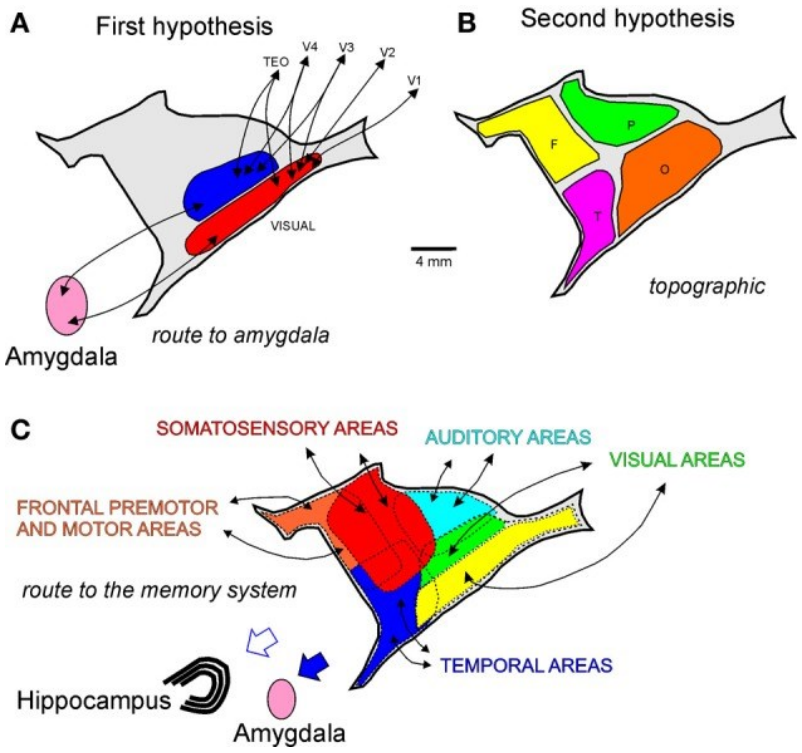

Fig. 11. Hypotheses on the organization of the claustral projections. Lateral reconstructions of the claustrum with pictorial representations of three connectional hypotheses: (A) route to the amygdala; (B) topographic (O, occipital; P, parietal; T, temporal; and F, frontal connections); and (C) route to the memory system (Gattass et al., 2014).

Specifically, they described the projections of the CLA to several visual areas and observed a segregation of the territories projecting to the different areas, suggesting the existence of a coarse visual topography in the CLA. Furthermore, based on comparisons of their data with those published in previous papers, they proposed three hypotheses for the connectional organization of the CLA. The first hypothesis is named "route to amygdala" because the two areas of visual organization are interconnected to areas of the visual cortex and project to the amygdala, which may contain information about objects in visual space. They suggest that the ventral CLA and mid CLA are gateways for the transfer of visual information to the memory system based on Turner et al. (1980) that showed these sections of the claustrum project to the amygdala. The second hypothesis considers a topographical organization of the CLA, in which projections to distinct neocortical lobules are topographically segregated. The last hypothesis suggests that

different parts of the CLA process different types of sensory information and is also based on tractographic data in humans by Milardi et al. (2013). In this latter view, the anterior dorsal region is more connected to the somato-motor areas, the lateral pathway connects the CLA to the auditory cortex, and the posterior ventral part to the visual-spatial oculo-motor circuits. More recently, Gamberini and colleagues described the projections from the CLA to the superior parietal cortex following injections of retrograde neuronal tracers in both anterior and posterior cortical areas, involved in visuo-motor processing based on visuo-spatial information for the control of arm and eye motor behavior (Gamberini et al., 2017, 2021). They found that the visuo-motor and multisensory areas of the superior parietal lobule receive projections from a wide region of the CLA, including also the anterior and ventral parts of the CLA, and suggested that neighboring cortical areas receive projections from different CLA territories, if involved in different functional processing.

The data of the present study, together with previous data from the same lab (Luppino et al., 2022) suggest a more complex view of the claustral organization, in which different parts of the CLA project to cortical areas and subcortical structures jointly involved in large scale functional networks, such as the lateral grasping network.

5.2 FUNCTIONAL ROLE OF THE CLAUSTRUM WITHIN THIS ORGANIZATION

As discussed, CLA has extensive connections with PFC, which is critically involved in higher order executive functions. Recent studies showed that in rodents the CLA has a predominantly inhibitory control over PFC. Indeed, Jackson et al. (2018) demonstrated, through optogenetic activation of the CLA, that claustro-cortical projections lead robust feedforward inhibition of excitatory pyramidal cells within the PFC. Furthermore, Liu et

al. (2019), found that the CLA negatively control the activity of the PFC and that it participates to the regulation of attention, attributing a key role of the CLA–PFC pathway in regulating impulsivity.

In rodents, the CLA seems to be also involved in the salience network, a circuit that elaborates the relevance of the stimuli. Specifically, through resting-state functional magnetic resonance imaging (rs-fMRI) in rats, it was observed that the CLA is part of the rodent homolog of the salience network (Smith et al., 2017). This network consists of connections between mPFC, MD, and CLA in the awake state. These areas are engaged by cognitive demanding tasks and regularly interpreted as constituting the *salience network* that contributes target brain regions in the generation of appropriate behavioral responses to salient stimuli. This network seems to be critical for guidance of thought and behavior (Seeley et al., 2007; Menon and Uddin, 2010). To test this hypothesis, studies using specific tasks and CLA suppression showed the involvement of CLA in cognitive processes (Jackson, 2020). Neuroanatomical tracing studies reveal the structural connectivity supporting the functional connections shown in the rs-fMRI in rats (Smith, et al. 2017, Jackson, 2020). Remedios et al. (2010), following experiments on awake primates, also suggested that the function of CLA is related to salience. They analyzed multisensory integration in CLA and concluded that it seems to play a role as a salience detector. In particular, it appears that CLA acts as a modulator and integrator of synchronized oscillations (Smythies et al., 2012). In this sense, CLA could play a role in attention and in binding feature. Vohn et al. (2007) examined fMRI response in three divided attention tasks into different sensory modalities. In cross-modal tasks, the main involved hemisphere was the right one, with activation in PFC, inferior parietal cortex and CLA, independently by the sensory modality was used, while in within-modal tasks the CLA was less involved. So, although the CLA has the largest global level of activation

in cross-modal tasks, it also exhibits considerable activation in the intra-modal task, suggesting that CLA has different modalities for integrative features and that is crucial for coordinating two activities at once, whether they are presented in the same sensory modality or two different ones. On the other hand, in focused attention tasks, CLA is associated with the activation of the higher visual and auditory cortices (Emrich et al., 2006). About binding, “illusory conjunctions” are also relevant (Crick and Koch, 2003; Treisman and Schmidt, 1982). When subject's attention is distracted or overloaded, often stimuli that vary in color and shape appear to be unnoticed and features can be mistakenly recombined, so subjects see illusory conjunctions in 50% of trials, as there was not enough time for claustro-striate iterations to give binding through attention. Smythies et al. (2012) argue that ascending brainstem afferents constitute the searchlight for binding in attention, designating the CLA as a saliency detection, which is based on the idea that CLA is involved in the binding features of sensory stimulus (Crick & Koch, 2003).

5.3 THE ROLE OF THE CLAUSTRUM IN COGNITIVE PROCESSES

As discussed above, the ventral part of the CLA has extensive connections with limbic areas, such as the anterior cingulate gyrus, the amygdala, the hippocampus, while its dorsal part has major connections with the sensory and motor cortices.

The role of CLA could therefore concern higher order functions, reinforcement, memory and limbic functions. In this view, CLA collaborates with the sensory cortex for sensory binding, with the limbic system both for emotional coordination and modulation of behavior through complex reinforcement learning and with PFC about decision-making mechanisms to coordinate higher-order functions. Concerning these latter functions, in order to map the usage of fluency heuristics onto certain brain regions, Volz et al. (2010) conducted fMRI studies, finding a relevant CLA activation. They tested the recovery

time of a long-term memory trace in healthy subjects, considering the subject's emotional component of memory, evaluating fMRI brain activation, with two hypoteses: the first one was that the VMPFC is a neural correlate of fluency-heuristic-based judgments, the second one was that CLA is a neural correlate of judgments based on fluency heuristics, which was developed from an account of retrieval fluency based on successfully binding memory traces. Their results show activation of dorsal CLA, but not vmPFC, suggesting that decisions based on fluency heuristics differ from metacognitive judgments about the perceptual accuracy of memory and that CLA can also bind semantic and emotional information, and the integration of perceptual and memory components into a conscious percept is considered to be reflected in the activation of the claustrum.

Another possible function that the CLA can perform concerns “insight”. The insight is the phenomenon for which the solution of a problem pops out suddenly. Tian et al. (2011), through fMRI, demonstrated that the insight process is related to the activation of the PFC and temporal areas, the cerebellum and the CLA bilaterally.

CLA activity also concerns the rapid interhemispheric transmission of information necessary for bilateral coordination of movement regulation (Smith and Alloway, 2010). According to the Smythies at al. (2012) hypothesis, the CLA would bind the two information coming from the corresponding left and right motor areas. In addition, CLA operates both globally and at a local level, integrating the activities generated by other circuits. Emrich et al. (2012) described a circuit involving the anterior cingulate gyrus for expectancy and saliency and PFC for higher mental functions including memory, as well as M1. In order to properly convey sensory inputs to motor output, the brain must have a system that connects rapidly different cortical areas and controls high order functions as attention and memory., Smythies et al (2012) suggest that the CLA, with its connections and circuit, may be the structure assigned to this role. In line with this,

Remedios (2012) conducted a lesion study in rats using saporin, a protein that was able to almost completely inactivate CLA. In the experimental situation, the rats were on an elevated eight-armed radial labyrinth: the inactivated rats were "frozen" in the center and did not explore as the control ones. Likewise, rats placed in a running situation on a Rotarod continued to run much longer than control subjects. Furthermore, using blood oxygen level-dependent (BOLD) fMRI in these aclaustral rats, Remedios reported broad positive correlation enhancements in various sensory cortical regions, as well as increased activity in the PFC. When a mechanism in the brain is disabled, it immediately tries to activate other mechanisms to balance the loss. In this case, loss of CLA integrative activity results in an expected compensatory increase in the activity of cortico-cortical circuits. About this study, Smythies et al. (2012) assert that behavioral or emotional "freezing" has nothing to do with the lesioned rat being "frozen" in the maze's center. The rat is unable to distinguish between two extremely identical arms of the labyrinth if the claustrum is lost. According to Smythies et al. (2012), competing synchronous gamma oscillations have an impact on sensory and much cognitive processing that results in executive orders. The link between the two is maintained through corticoclaustro-cortical loops. This mechanism functions at a weak level inside the cortex by cortico-cortical synchronizations, and at a greater level within the claustrum, via intraclaustral synchronizations. The remaining cortical signals are insufficient to give the command necessary for the rat to choose one arm of the labyrinth. So, the injured animal can no longer make a decision and, similarly, the injured animal running, by activating a similar mechanism, cannot decide when to stop.

6. CONCLUSION

This study shows a large overlap between the territories of the CLA that project to different cortical areas and to the related subcortical structures in the macaque, and thus the involvement of different claustral parts in different large-scale functional networks. The connectional organization suggests a role of the CLA in coordinating the activity of distant cortical areas and subcortical structures within the same functional network. This function of the CLA could be based on the activation of inhibitory neurons in the cortex, as suggested by different rodent studies.

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