Eur J Anat, 2 (2): 115-122 (1998)

Subdivisions of the human substantia nigra using AChE histochemistry

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Summary

The present work describes the neurochemical activity detected in the nigral complex of the adult human using the acetylcholinesterase histochemical technique. Such activity is seen at cell level in the pigmented neurons of the pars compacta of the substantia nigra, and, to a lesser degree, in the paranigral neuronal groups (ventral tegmental area and retrorubral field). There is also considerable enzymatic activity in the neuropil of the substantia nigra. However, the pars lateralis and overall the ventromedial portion of the substantia nigra present a relatively low acetylcholinesterase content, though both have some acetylcholinesterasic neuronal bodies. A series of studies in different species suggested that in the substantia nigra, AChE is released almost exclusively from dopaminergic nigrostriatal neurons and precisely from their dendrites, which protrude widely into de pars reticulata. In addition, previous findings made in the cat and in the monkey reported a direct correlation between nigral AChE activity and nigrostriatal connections. The non-uniform distribution of AChE activity within the human nigral complex reported here may indicate the modular organization of the intrinsic neurons of the substantia nigra and their mesostriatal projections.

Key words: Acetylcholinesterase - substantia nigra - human - histochemistry - basal ganglia

Introduction

The substantia nigra (SN) is a region within the ventral mesencephalon involved in the regulation of motor function. From a cytoarchitectural point of view, the human substantia nigra, like that of other mammals, can be divided into a *pars compacta* (SNc), situated dorsally in the cerebral peduncle, and a *pars reticulata* (SNr), situated ventrally (Olszewski and Baxter, 1954). The latter, however, is invaded by some neurons of the SNc and, above all, by dendrites of these SNc neurons. At the lateral edge of the SN, the so-called *pars lateralis* (SNl) can be distinguished, although this subdivision is usually considered to form part of the SNc (Poirier et al., 1983). Closely related with the substantia nigra is a series of paranigral neuronal groups that constitute on the one hand the ventral tegmental area (ATV), located medial and dorsal to the substantia nigra, and, on the other hand, the retrorubral fields (Rr), located dorsal to the SN, in its caudal and lateral half (Bogerts, 1981). The dopaminergic neurons of these nuclei, like those of the SNc, which form the so-called mesencephalic dopaminergic groups A8 (Rr), A9 (SNc), and A10 (ATV) (Dahlström and Fuxe, 1964), are the origin of the mesostriatal and mesolimbocortical projection systems, supplying dopaminergic innervation to the striatal body and to other brain structures (Llamas and Reinoso-Suárez, 1969; Fallon and Moore, 1978; Swanson, 1982; Deutch et al., 1984), and are involved in the pathophysiology of Parkinson's disease and other extrapyramidal disorders. In man and other higher primates, these neurons contain neuromelanin, a product derived from

Submitted: November 13, 1997
Accepted: July 10, 1998

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dopamine metabolism, which is a splendid natural neuronal marker, making these cells visible without the need for immunohistochemical detection (Bogerts, 1981; Saper and Petito, 1982). In addition, the vast majority of dopaminergic neurons contain acetylcholinesterase (AChE) (Butcher et al., 1975). In turn, additionally the SNr receives its major inputs from the striatum, and contains GABAergic neurons that project to the thalamus, superior colliculus and the reticular formation (Parent and Hazrati, 1995).

The study of chemoarchitecture is important in the delineation of different areas of the brain. The use of chemoarchitecture in the human brain becomes even more significant because the connectivity criterion is virtually impossible to apply (Huang et al., 1993). Fortunately, a striking resemblance in the neurotransmitter or neuromodulator content of nuclei has been observed across species (Paxinos et al., 1995).

Many studies have demonstrated that, as in other species, the human substantia nigra is by no means an homogeneous structure in neurochemical terms (e.g., McRitchie and Halliday, 1995). In the rat (Weston and Greenfield, 1986), the release of AChE from the substantia nigra seems to be mainly dependent on intrinsic nigrostrioatal projection neurons, and occurs chiefly from their dendrites. It has thus been postulated that liberation of AChE from the nigrostriatal system is more related to dopamine transmission rather than to cholinergic systems (Greenfield, 1984). Although previous studies in the human mesencephalon using AChE histochemistry have not reported any kind of unhomogeneity in the SN (see, for instance, Paxinos et al., 1990; Paxinos and Huang, 1995), similar studies carried out in other animal species such as the cat (Jiménez-Castellanos and Graybiel, 1987a) and the squirrel monkey (Jiménez-Castellanos and Graybiel, 1987b) have shown the differential distribution of AChE activity in the nigral complex, and the direct relation of such histochemical activity to nigrostriatal connections. Given these facts, the aim of the present work was to study the possible histochemical compartmentalization of the human nigral complex as revealed by the AChE technique.

Material and Methods

We used the encephalons of three white individuals who died without known neurologic or psychiatric deficits (case 1, a 65 year-old man; case 2, a 70 year-old woman and case 3, a 47 year-old man). Observations were made on immersion-fixed post-mortem human mesencephalic tissue. Time elapsed between death and tissue fixation was 22-32 hours. Brainstems were cut into 2 blocks and exposed successively to (i) fixative solution [4% (wt/vol) paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.4] for 24 hr, and (ii) a series of wash solutions [0.1 M sodium phosphate buffer containing, consecutively, 0, 5, 10, 15 and then 20% (wt/vol) sucrose, each for 12 hr]. Frozen sections were cut at 40 μm in the transverse plane on a sliding microtome and collected successively in a compartmentalized box, to obtain 12 equivalent series of sections.

Successive compartments were processed according to (a) the Nissl method; (b) the Heidenhain-Woelcke myelin stain (Grüber, 1981); and (c) a modified Geneser-Jensen and Blackstad (1971) technique for localizing AChE activity (Graybiel and Ragsdale, 1978), in which sections were developed in potassium ferricyanide following incubation in acetylthiocholine iodide solution containing the pseudocholinesterase inhibitor, ethopropazine. As a control, some sections were processed following the AChE technique, but without adding acetylthiocholine iodide to the incubation solution. In these cases, no marking was observed either at neuronal level or in the neuropil.

Sections were studied with bright-field illumination, and were charted onto drawings made with the aid of an overhead projector. To explore the expected histochemical heterogeneity, we carried out serial-section comparisons of the nigral architecture shown in sections stained for Nissl substance, myelin stain, and AChE.

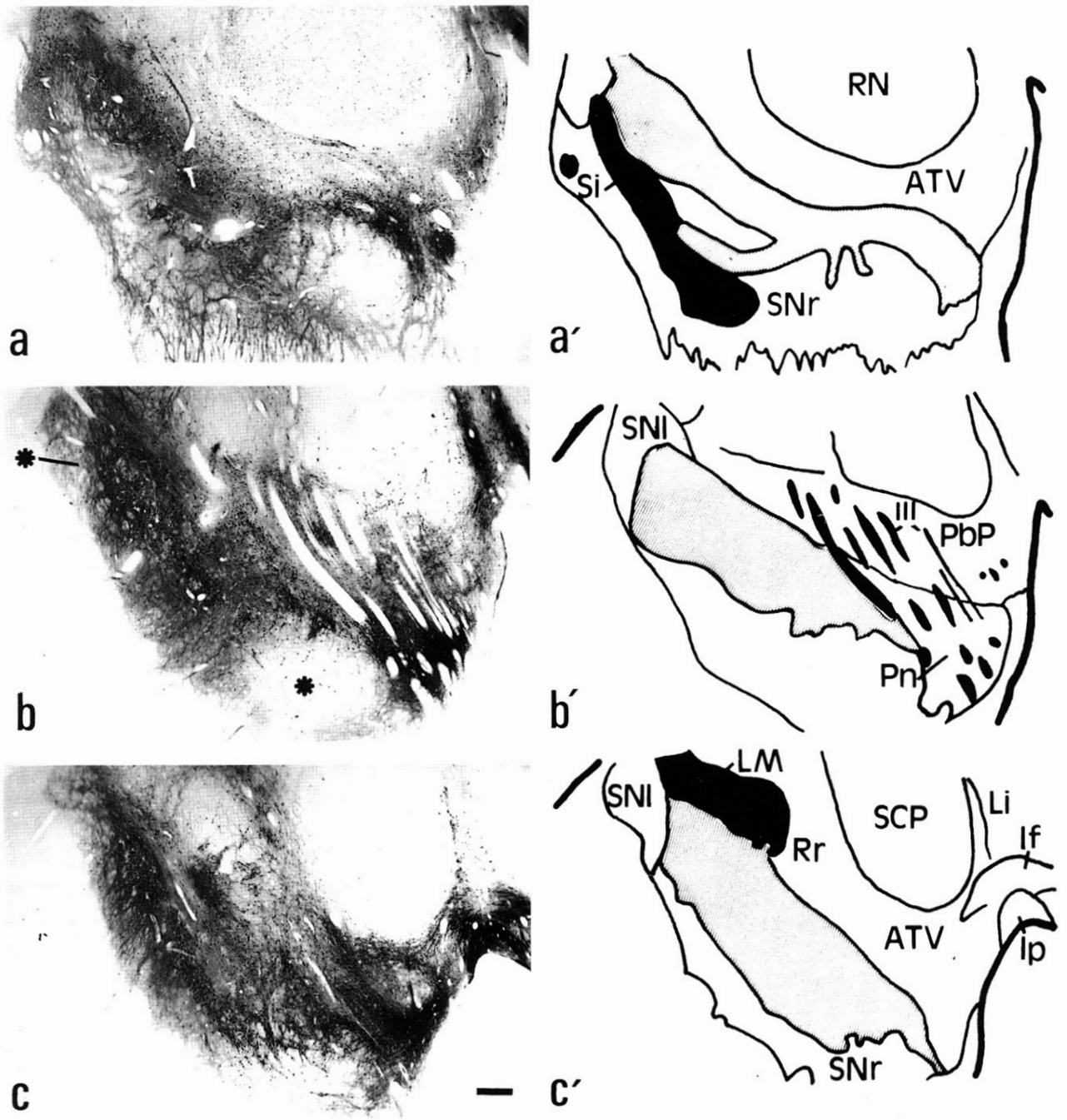

Fig. 1.– Case 1. On the left (**a**, **b**, **c**), light-field photographs of craniocaudally ordered transverse sections through the human nigral complex of the right side, showing AChE activity. On the right (**a'**, **b'**, **c'**), their corresponding diagrammatic chartings, indicating the main architectural subdivisions of the nigral complex. The SNc, which generally shows the highest (neuronal and fibrillar) AChE staining, is represented by shaded areas. With the exception of the paranigral nucleus, the ATV and the retrorubral nucleus dysplay a moderate staining in the neuropil, though both contain AChE neuronal bodies. Asterisks indicate nigral zones with a relatively low AChE activity in the neuropil. The light staining of the neuropil is particularly evident cranio-caudally in the ventromedial region of SN. Abbreviations: ATV, ventral tegmental area; If, interfascicular nucleus; Ip, interpeduncular nucleus; Li, linear nuclei of the raphe; LM, medial lemniscus; PbP, parabrachial pigmented nucleus; SCP, decussation of the superior cerebellar peduncle; Pn, paranigral nucleus; RN, red nucleus; Rr, retrorubral fields; Si, stratum intermedium (fibres); SNl, pars lateralis of SN; SNr, pars reticulata of SN; III, oculomotor nerve fibres. Calibration bar = 1mm.

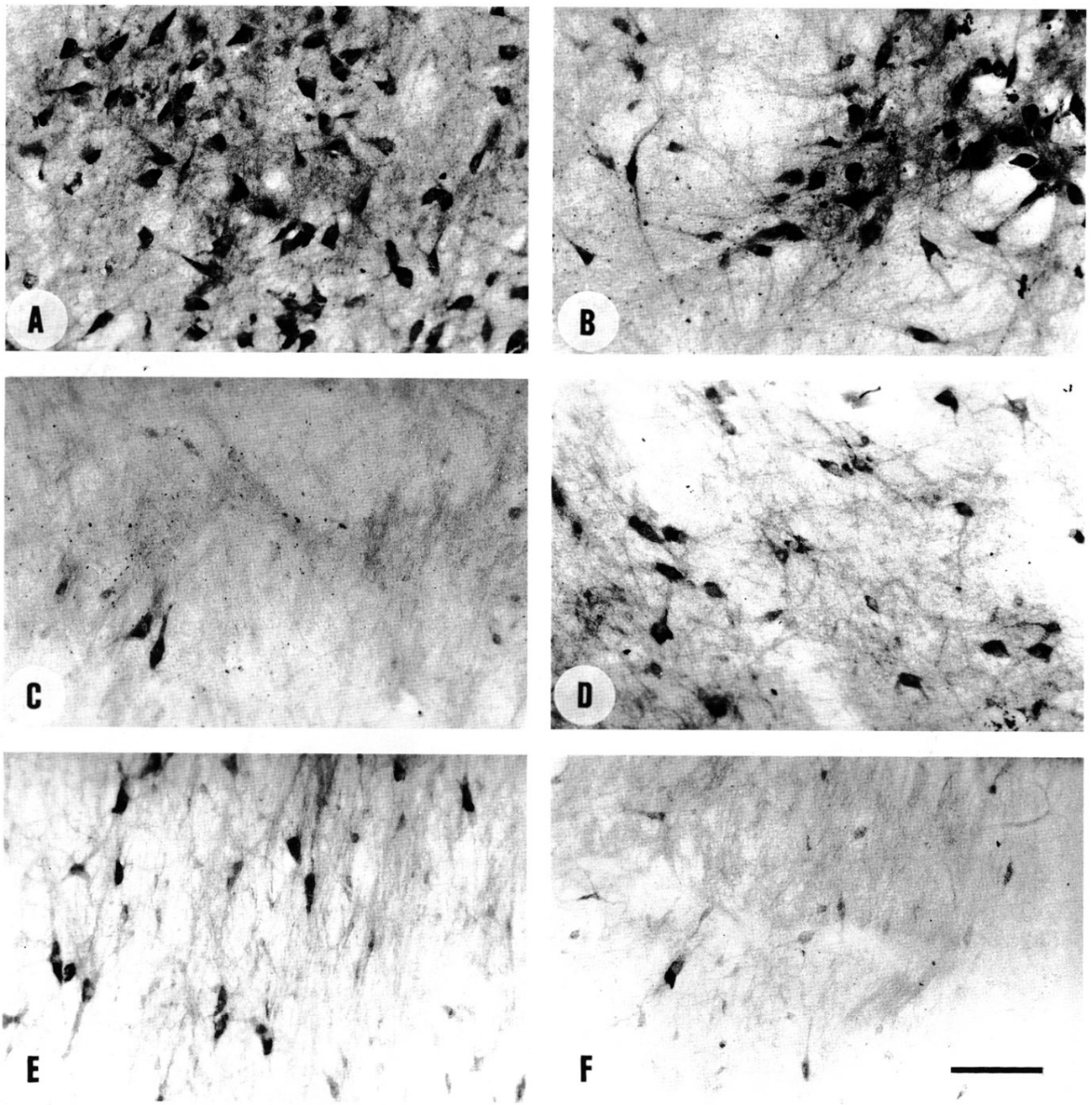

Fig. 2.– Higher power photomicrographs showing AChE activity in different regions of the nigral complex in case 2. (**A**, **B**) Relatively high AChE content in neurons and neuropil of SNc; a digitation of SNc protruding into the AChE-poor ventromedial region of SN is seen in B. (**C**) Relatively low AChE activity in the neuropil of the ventromedial zone of SN where some labeled neurons are detected. (**D**, **E**) Intermediate intensity of AChE staining observed in the neuropil of the lateral ATV and in Rr, respectively. Some AChE–positive neuronal bodies are seen in both regions. (**F**) Relatively low AChE content in SNl. Some AChE –positive neuronal bodies are seen in the midst of a fibrillar rich neuropil mostly devoid of AChE staining. Calibration bar = 0.2 mm.

Results

The AChE activity detected in the nigral complex was comparable in all cases, both at cell level and in the neuropil.

A) *AChE activity at neuronal body level* (Fig. 1).

In the SN, AChE activity at neuronal soma level was confined to the SNc, where, as is well known, the dopaminergic neurons also contain AChE. The distribution of acetylcholinesterasic neurons was therefore similar to that of the neuromelaninergic neurons that were seen in adjacent sections stained for Nissl. While neurons were plentiful in the medial and lateral portions of the SNc (Fig. 2 A), some AChE-containing neurons invaded the subjacent SNr, forming digitiform prolongations (Fig. 2 B), or as isolated neurons. The SNl also contained few acetylcholinesterasic neurons (Fig. 2 F).

No AChE activity was observed at somatic level in the SNr, apart from that already mentioned, but numerous AChE-positive neuronal bodies were found in the ATV (Fig. 2 D). These were especially abundant in certain subdivisions, such as the paranigral nucleus (situated medial to the SNc, Fig. 1), and were more disperse in other subdivisions of this formation of the nigral complex. Some acetylcholinesterasic neurons were observed in the retrorubral nucleus (Fig. 2 E).

B) *AChE activity in the neuropil* (Fig. 1).

With the methodology used, we were unable to detect minor differences between the intensity of staining of the different regions of the nigral complex. However, the AChE activity detected in the neuropil of the nigral complex was unequal and different levels of staining (high, intermediate and low) were observed.

In the substantia nigra, both the SNc and the SNr showed enzymatic content in the neuropil. Most of these nigral subdivisions showed a relatively high AChE content (Fig. 2 A). However, some nigral regions were characterized by a relatively low AChE activity. The most notable were the lateral edge of the SN (presumably part of SNl because of its fibrillar richness, Fig. 2 F) and, above all, the ventromedial region of the SN (Fig. 2 C), which, craniocaudally (Fig. 1), showed a relatively low AChE activity.

The enzymatic content was also unequal in the neuropil of the ATV. At rostral and caudolateral levels, the staining intensity seen in ATV (Fig. 2 D) was intermediate between those of the zones showing high and low AChE content detected in the substantia nigra. At more caudal levels of ATV, especially in the medial region corresponding to the paranigral nucleus, the intensity of staining was similar to that in the regions of the SN showing a relatively high AChE content.

Finally, the neuropil of the retrorubral nucleus (Fig. 2 E) showed an intermediate AChE staining similar to that of the adjoining caudolateral regions of the ATV.

Discussion

The use of postmortem human encephalic material when investigating the distribution of different neurochemical compounds is complicated by alterations occurring as a result of death. However, the carboxylic hydrolases, and specifically AChE, seem to be very stable in postmortem material, even up to 40 hours after death (Biegon and Wolff, 1986). This fact, together with the total absence of neuronal and fibrillar staining for AChE in our controls, confers a high level of confidence to the validity of our findings.

We believe that the histochemical technique of AChE, despite already being classic, retains its interest due to (among other features) the confirmed relationship at SN level between this enzyme and the neurotransmitter dopamine. Like dopamine, AChE is known to be liberated in the rat striatum by nigrostriatal afferents (Greenfield et al., 1983). A series of studies in the rat and in the guinea pig (Henderson and Greenfield, 1984; Rotundo, 1984) suggests that in the substantia nigra, AChE is released almost exclusively from dopaminergic nigrostriatal neurons and precisely from their dendrites which protrude widely into the SNr (Weston and Greenfield, 1986), where liberation of dopamine also takes place (Wassef et al., 1981). This assertion is based on the fact that in the SN of the rat there appear to be no dopaminergic axon terminals or collaterals (Wassef et al., 1981). Why nigrostriatal dopamine neurons contain AChE is open to speculation. In principle, nigral AChE could inactivate the acetylcholine liberated by some extrinsic afferent cholinergic projection system. The major candidate as origin of a cholinergic projection to the SN is the pedunculopontine tegmental nucleus. However, the existence, relative importance, and distribution of a cholinergic pedunculopontine-nigral projection in the rat is still controversial, and different researchers disagree (Beninato and Spencer, 1988; Gould et al., 1989; Martínez-Murillo et al., 1989; Fujimoto et al., 1990). Another attractive possibility, supported by studies in the rat (Weston and Greenfield, 1986), is that dendritic liberation of AChE is related to dopamine release, both compounds acting as neuromodulators in the substantia nigra.

Could the unequal distribution of AChE activity that we have observed in the human nigral complex be related to a particular organization of the intrinsic neurons and/or to their projections? This is a question outside the aim of this study. Moreover, there is no available neuroanatomical tracer that "in vivo" may be injected into the human brain to study the connections of a particular CNS structure. However, we can proceed by comparing our results in the human brain with those coming from animal experimentation, where a combination of AChE histo-

chemistry and neuroanatomical tracing techniques can be achieved.

From experiments carried out in the rat (Butcher et al., 1975; Lehman and Fibiger, 1979), it is well known that most –if not all– dopaminergic neurons of the SN also contain AChE. However, to our knowledge there are no reports describing a non-uniform distribution of AChE activity in the SN of the rat. The opposite occurs in other animal species. Thus, both in the cat (Jiménez-Castellanos and Graybiel, 1987a) and in the squirrel monkey (Jiménez-Castellanos and Graybiel, 1987b), zones of the SN were characterized by their relatively low content of AChE next to other AChE-rich nigral subdivisions. In both species, as in the human, the reduced enzymatic activity was seen mainly in the neuropil, even though AChE-positive neuronal bodies could be seen not only in AChE-rich zones but also inside or in the immediate neighborhood of the AChE-poor zones.

The AChE compartmentalization of the SN has been shown to be a reflection of the differential organization of the mesostriatal projection systems which originate in the dopaminergic neurons of the nigral complex. Thus, in the cat (Jiménez-Castellanos and Graybiel, 1987a) the caudomedial portion of the SNc (densocellular zone), which corresponds to the only AChE-poor region of the nigral complex, specifically innervates AChE-poor zones of the striatum called striosomes (Graybiel and Ragsdale, 1978). The SNl also innervates striosomes located at caudoventral regions of the caudate nucleus of the cat. In contrast, the rest of the SNc, the ATV, and the A8 dopaminergic cell group (roughly equivalent to the retrorubral fields)- all zones showing a relatively high AChE-content- preferentially innervate the extrastriosomal matrix compartment of the striatum, characterized by its higher AChE activity. Similarly, in the squirrel monkey (Jiménez-Castellanos and Graybiel, 1989a; Feigenbaum et al., 1991), tracer deposits made in the ventral digitations of the SNc which protrude into the SNr- regions which show a reduced AChE activity- gave rise to a fibrillar labeling of striosomes. The extrastriosomal matrix in contrast was labeled by anterograde tracer injections centered in AChE-rich regions of the nigral complex.

In comparison with the findings referred to above, the results reported here suggest that the AChE compartmentalization of the human SN could represent the modular organization of the intrinsic neurons of SN in terms of their differential projection systems. Given the fact that the striosomal compartmentalization of the striatum is present in all mammals (including man), an attractive possibility is that AChE nigral subdivision could indicate the existence of different channels of mesostriatal innervation of striosomes and extrastriosomal matrix of the striatum or even other subdivisions present in the striatum, like the "matrisomal" compartmentalization detected in the cat (Jiménez-Castellanos and Graybiel, 1989b; Kemel et al., 1989). This hypothesis could be of interest from a functional point of view since the striosomal compartment of the striatum has been considered to be related mainly to the modulation of cognitive or limbic aspects of the function assigned to the basal ganglia, whereas the extrastriosomal matrix would be concerned mainly with the regulation of more purely sensorimotor functions (Gerfen, 1992).

A set of studies has shown the differential distribution of many neurochemical compounds within the boundaries of the human nigral complex. These substances include melanin (Bogerts, 1981), substance P (Mai et al., 1986), tyrosine hydroxylase (Pearson et al., 1990), met-enkephalin (Gaspar et al., 1983), calbindin (Hirsch et al., 1992; McRitchie and Halliday, 1995), and cytochrome oxidase activity (Vila et al., 1997), among others. These substances or activities were studied in many cases in both normal and pathological brains. Although the various nigral complex subdivisions proposed in these studies generally do not correspond from one study to the next, many authors suggest that the different content of a particular substance at neuronal and/or fibrillar level could be responsible for the differential vulnerabilities shown by dopaminergic neurons to pathology, especially in relation to Parkinson's disease. With regard to this point, the nigral region which shows the greatest neuronal loss in parkinsonian brains is the ventral tier of the SNc (Fearnley and Lees, 1991), a region that would include our ventromedial AChE-poor zone.

In conclusion, our results add new proof of the known heterogeneity of the human SN. For the first time an AChE nigral compartmentalization has been detected in the human mesencephalon. Other studies using different techniques will be necessary to confirm the suspected functional and even pathological implications of our present findings.

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