Histol Histopath (1991) 6: 373-380

Histology and Histopathology

Pathological changes in dendrites of substantia nigra neurons in Parkinson's disease: a Golgi study

Stephan Patt[1], Hermann-Josef Gertz[2], Lieselotte Gerhard[3], and Jorge Cervós-Navarro[1]

[1]Institute of Neuropathology, [2]Department of Gerontopsychiatry, Free University of Berlin, Berlin and [3]Institute of Neuropathology, University of Essen, Essen, Germany

Summary. Neurons of the substantia nigra show severe morphological changes in Parkinson's disease. Pathological alterations of cell bodies have been described, whereas those of neuronal processes have hardly been investigated. Golgi impregnation has been the chosen method for demonstrating neuronal processes and dendritic and somatic spines. We therefore used the Golgi-Braitenberg method to qualitatively and semi-quantitatively study the substantia nigra of eight patients with Parkinson's disease compared with eight control cases. Golgi impregnation of substantia nigra neurons was good in all control cases. In full agreement with the analysis of Braak and Braak (1986) three neuronal types within the substantia nigra were found. In cases of Parkinson's disease, severe pathological changes such as decrease of dendritic length, loss of dendritic spines and several types of dendritic varicosities were found only in the melanin-containing pars compacta neurons. Pars reticulata nerve cells were intact. These findings support the predominant role played by the dopaminergic efferent pathway in the degenerative process. The afferent pathway was not affected. This suggests that the substantia nigra lesion is primary in Parkinson's disease.

Loss of neurons found in H & E sections corresponded to a lesser amount of impregnated pars compacta neurons in cases with Parkinson's disease when compared to controls. Evidences exist that the duration of the disease may be related to the extent of pathologically altered Golgi-impregnated pars compacta cells. The amount of Lewy bodies in H & E sections corresponded to the quantity of round varicosities in impregnated pars compacta neurons. These round dendritic varicosities were considered to be Lewy body inclusions. They seem to have no influence on the dendritic spine density and morphology in most cases.

Offprint requests to: Dr. Stephan Patt, Institut für Neuropathologie der Freien Universität Berlin, Hindenburgdamm 30, D-1000 Berlin 45, Germany

Key words: Substantia nigra, Parkinson's disease, Golgi technique

Introduction

Neurons of the nucleus niger undergo severe morphological changes in connection with age-associated diseases impairing motor functions, e.g. Parkinson's disease (Hassler, 1938).

Gross inspection as well as microscopic investigation show a severe loss of neuromelanin-containing nerve cells of the substantia nigra. Details of the normal histology of the nucleus niger were described by Bauer (1909), Hassler (1938) and Olszewski and Baxter (1954) on the basis of studies in paraffin-embedded and routinely stained material. Braak and Braak (1986) performed a detailed pigmentoarchitectonic analysis of the black nucleus using a special deimpregnation technique (Braak, 1983).

This study is a qualitative and semi-quantitative Golgi-impregnation analysis of the pathologically changed nucleus niger. Golgi-impregnated neurons of the black nucleus of elderly patients suffering from Parkinson's disease were compared with those of human adults without Parkinson's disease. The present study focuses on the pathology of dendrites, which can be excellently visualized by means of the Golgi technique. Furthermore, this study demonstrates what types of substantia nigra neurons are affected in the degenerative process.

Materials and methods

The brains of eight adults without Parkinsonian symptoms, ranging in age from 46 to 87 years (mean : 60 years), served as controls. They were compared with the brains of eight patients with Parkinson's disease, who ranged in age from 64 to 82 years (mean : 71 years). Tables 1 and 2 show clinical and pathological data

of control and Parkinson cases. The brains were obtained at autopsy, fixed in toto in 10% formaldehyde and stored for at least six months. The time between death and autopsy was at least one day and, in two control cases, up to five days. For Golgi impregnation, the brain stem was cut in frontal sections. Blocks of 1 x 1.5 x 1 cm containing the nucleus niger were selected for the Golgi technique and processed according to the modification proposed by Braitenberg et al. (1967). The blocks were frozen, cut at 100 μm, quickly dehydrated and mounted in synthetic resin (Eukitt). Approximately 20 sections of each case were examined. Well-impregnated neurons were photographed at different magnifications. Montages of photomicrographs at various depth of focus were made. Some of the neurons were additionally rawn using a camera lucida.

Routinely stained paraffin sections of the substantia nigra were available for conventional examination.

Results

Controls

The quality of Golgi impregnation was similar in

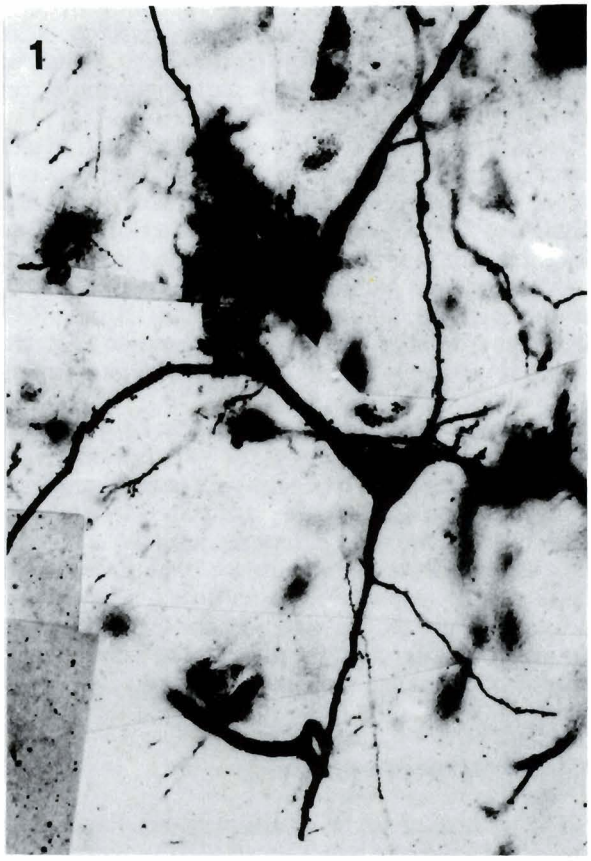

Fig. 1. Golgi-impregnated type I neuron of the substantia nigra pars compacta (control case, × 250). The perikaryon is triangular and generates primary dendrites whose proximal course is unbranched. The dendrites show rather short spines. No axon is visible.

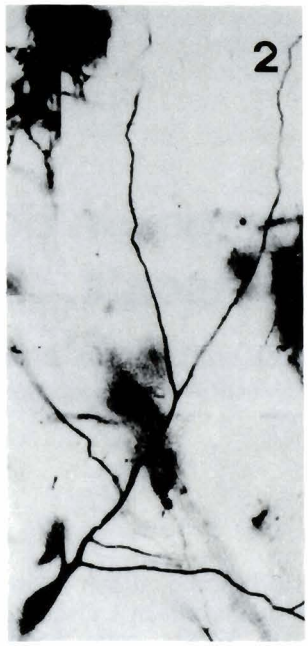

Fig. 2. Golgi-impregnated type II neuron within the substantia nigra pars reticulata (control case, × 250). The dendrites are smooth. Their main segments are devoid of spines.

Fig. 3. Golgi-impregnated type III neuron of the substantia nigra pars compacta (control case, × 250). Three rather short dendrites arise from the small fusiform perikaryon. Delicate filiform processes are found in the distal portions of the dendrites (arrows).

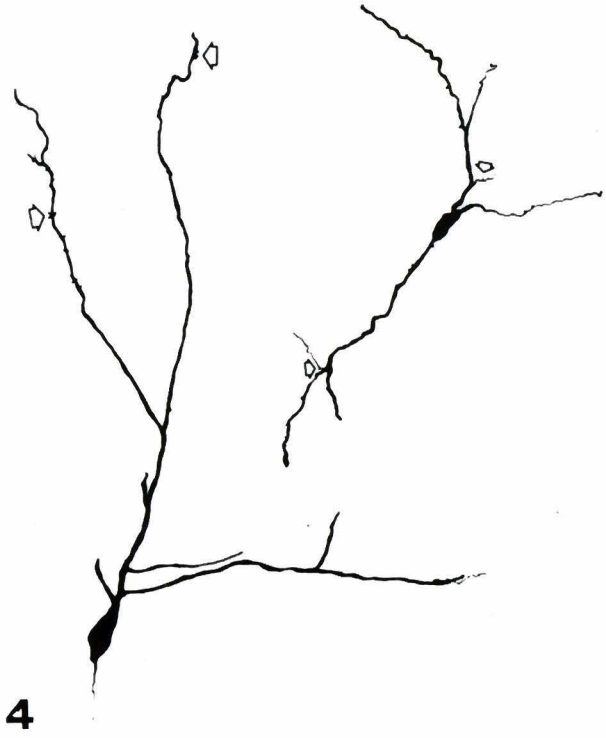

Fig. 4. Camera lucida drawings of a type II neuron and a type III neuron (see Figs. 2 and 3). Note the characteristic spine distribution (arrows) in the type II neuron and the delicate filiform processes in the type III neuron (arrows).

Fig. 5. Three Golgi-impregnated type I neurons in the substantia nigra pars compacta (Parkinson's disease, × 300). Two neurons have only short shrunken primary dendrites and resemble cell residues. Their perikaryon is enlarged. No somatic or dendritic spines are seen.At the top, one rather well-preserved type I neuron with long dendrites.

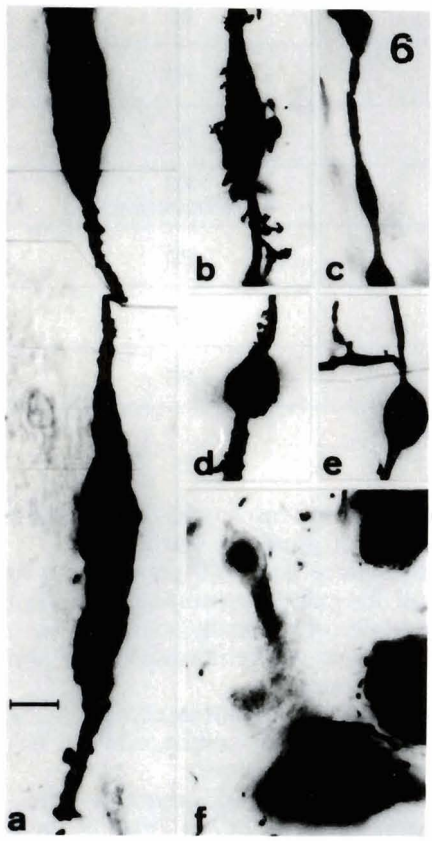

Fig. 6. Dendrites of type I substantia nigra neurons. (a-e, Golgi impregnation, Parkinson's disease, × 750). **a.** type A varicosities (bar = 20 μm). **b.** fusiform type B varicosity with spines. **c.** small fusiform type D varicosities. **d.** type C varicosity. **e.** type C varicosity, dendritic branches. **f.** Dendritic Lewy-body inclusion in a neuromelanin-containing locus coeruleus neuron (Palmgren silver stain, Parkinson's disease, × 750)

Fig. 7. Golgi-impregnated type I and type II neuron of the substantia nigra (Parkinson's disease, × 120). The type I neuron (1) in the pars compacta is severely damaged, while the type II neuron (2) in the pars reticulata is well preserved and shows long dendrites.

all cases. Well-known limitations regarding the Golgi technique itself were also observed here. The Golgi-Braitenberg variant used in this study generally yielded good results in providing a clear demonstration of spines, though dendritic and somatic contours were sometimes irregular and showed small varicosities. Cells consisting only of a swollen perikaryon and short shrunken primary dendrites were seldom found.

In agreement with the analysis of Braak and Braak (1986), three types of Golgi impregnated neurons could be differentiated within the borders of the nucleus niger.

Medium-sized fusiform, ovoid or pyramidal *type I neurons* (Fig. 1) were localized mainly in the pars compacta and only occasionally in the pars reticulata. Their cell body had an average length of 50 μm. Wide intraindividual variation was observed in the branching pattern of the dendrites. Spine density was variable. A mean number of approximately ten to twenty type I neurons per section was impregnated (Table 3).

Smaller *type II neurons* with cell bodies of an average length of 30 μm were primarily found in the pars reticulata. The main dendritic segments were characteristically devoid of spines, whereas the thin terminal portions often showed them in isolated clusters (Figs. 2, 4). Type II neurons were rarely impregnated. Only an average number of one to three cells could be observed in each section (Table 3).

Small *type III neurons* with 20 μm in cell body diameter (Fig. 3) could extremely rarely be found in both the pars compacta and the pars reticulata. Mainly three thin dendrites emerged from the perikaryon. The distal dendritic portions characteristically gave rise to axon-like delicate

Table 1. Clinical and pathological data of control cases (C)

Case	Sex	Age at death (years)	Clinical diagnosis	Cause of death	Neuropathological diagnosis*
C1	M	46	Cerebral ischemia	Acute ischemia	Cerebral infarction
C2	M	47	Spinal cord trauma	Trauma	Severe brain edema Traumatic spinal cord
C3	F	51	Neurosis	Suicide	No pathology
C4	F	52	Schizophrenia	Suffocation	No pathology
C5	F	56	Meningoencephalitis	Pulmonary embolism	Meningiosis carcinomatosa Stomach cancer
C6	F	66	Cerebral tumor	Tonsillar herniation	Glioblastoma
C7	F	71	Psychosis	Pulmonary embolism	No pathology
C8	F	87	Cerebral trauma	Pneumonia	Subarachnoidal hemorrhage

* Substantia nigra wihout pathological changes

Table 2. Clinical and pathological data of cases with Parkinson's disease (PD)

Case	Sex	Age at death (years)	Clinical diagnosis	Cause of death	Neuropathological diagnosis
PD1	M	64	Pick's disease Dementia	Pneumonia	PD
PD2	M	69	Severe PD Organic brain syndrome*	Pneumonia	PD
PD3	F	69	PD* Ileus	Bleeding from gastric ulcer	PD
PD4	M	70	PD*	Trauma	PD
PD5	F	70	PD* Temporal infarction	Pneumonia	PD Cerebral infarction
PD6	M	75	PD* trauma	Pneumonia	PD
PD7	F	75	PD*	Cardiovasculatory failure	PD
PD8	M	82	PD*	Cardiovasculatory failure	PD

* No dementia

filiform processes which displayed small varicosities and terminated in a rounded knob (Fig. 4).

Cases with Parkinson's disease

In all but two cases investigated, severe changes were found in *type I neurons*. The cell body was distended. The dendrites were reduced in length and often widened. Sometimes only short stumps of primary dendrites remained (cell residues - Fig. 5). Their endings were thickened in many cases.

Besides reduction of dendritic length and spine loss, the main findings in cases with Parkinson's disease were four types of dendritic varicosities.

Type A varicosities were large (40 μm or more in length), fusiform and irregularly contoured (Fig. 6a). They were often multiple, occasionally solitary. There was frequent connection of adjacent varicosities, and hardly any spines were found.

Type B varicosities were medium-sized (25-35 μm in length), fusiform and smoothly contoured (Fig. 6b) and sometimes bore elongated spines.

Type C varicosities were round, smoothly configurated and of variable size (Fig. 6d, e). Most of them

Table 3. Nerve cell loss examined in H&E sections compared to the mean number of Golgi impregnated type I, type II and type III neurons per section of the substantia nigra of control cases (C) and cases with Parkinson's disease (PD) compared to the duration of the disease. Statistical evaluation was not performed because of a low number of cases. In most PD cases, a long duration of the disease corresponded to a moderate to severe cell loss and to a low number of impregnated type I neurons.

Case	Cell loss	Type I neurons	Type II neurons	Type III neurons	Duration of PD (years)
C1	–	10	1	*	***
C2	–	25	3	1**	***
C3	–	20	1	*	***
C4	–	22	1	*	***
C5	–	17	1	*	***
C6	–	17	1	*	***
C7	–	10	2	*	***
C8	–	15	1	*	***
PD1	+++	4	1	*	several
PD2	++	4	2	*	2
PD3	+	5	1	*	several
PD4	+	30	4	1**	several
PD5	+	8	1	*	several
PD6	–	25	3	1**	1
PD7	+++	3	1	*	several
PD8	++	5	1	*	several

* absent
** per case only one cell found
*** not relevant here
– no pathological cell loss
\+ slight cell loss
++ moderate cell loss
+++ severe cell loss

were small (20-25 μm) and devoid of spines. Only some varicosities were covered with a few spines. In most cases, the dendritic segments adjacent to the type C varicosities were not altered. Only in case PD4, distally to a type C varicosity, did a neuron have thinner dendritic portions and increased and more elongated spines than in its dendritic portion between the varicosity and the perikaryon. Type C varicosities occurred more often in cases with high numbers of Lewy bodies observed in H & E sections (Table 4).

Type D varicosities (Fig. 6c) measured 10-25 μm in length and were often not clearly distinguishable from each other.

Varicosities were found in type I neurons of most cases (Table 4). The varicosities did not show any predilection with respect to the site. Type A and B occurred at multiple sites in one single cell. Type C varicosities were always solitary per one cell. In most cases, spine density on the dendrite appeared to be independent of the presence or absence of varicosities.

No gross alterations were seen in *type II neurons* (Fig. 7) except a slight reduction in dendritic extent and the appearance of some type D varicosities.

Type III neurons were extremely rarely impregnated in cases with Parkinson's disease. Thus they were not taken into account in this study. The few cells

Table 4. Comparison between amount of Lewy bodies observed in H&E sections, the occurrence of dendritic varicosities in type I nigra neurons and total amount of impregnated type I neurons per case with Parkinson's disease (PD). Relatively large amounts of Lewy bodies partly corresponded to high amounts of type C varicosities and large numbers of impregnated type I neurons.

Case	Lewy bodies	Type A var.	Type B var.	Type C var.	Type D var.	Type I neurons
PD1	++	–	–	++	++	+
PD2	?	–	–	–	+	+
PD3	++	–	+	+	+	+
PD4	++	+++	+++	++	+	+++
PD5	+	+	+	+	+	+++
PD6	+++	–	+	+++	+	+++
PD7	?	–	–	–	+	+
PD8	++	–	–	+	+	+

– absent
? Lewy body-like inclusions
+ few
++ several
+++ many

Table 5. Proportion of normal and abnormal cells per impregnated type I, type II and type III neurons in cases with Parkinson's disease (PD)

Case	Type I neurons	Type II neurons	Type III neurons
PD1	C	A	–
PD2	C	A	–
PD3	C	A	–
PD4	B	A	A
PD5	C	A	–
PD6	A	A	A
PD7	C	A	–
PD8	C	A	–

A approximately all impregnated neurons were normal
B approximately 50% of all impregnated neurons were normal
C approximately all impregnated neurons were abnormal
– no impregnated cells (see Table 3)

observed did not show any marked morphological changes.

Cell loss in H & E sections corresponded to a lesser amount of impregnated type I neurons (Table 3). With the exception of cases PD4 and PD6, impregnated type I neurons were significantly reduced in number as compared to the control cases (Table 3). On the other hand, amounts of impregnated type II and type III neurons were similar in both groups.

Case PD4 with normal quantities of type I neurons had Parkinsonian symptoms for several years, whereas the second case with a normal number of impregnated type I neurons (PD6) showed a short duration of the disease. The other case with a short duration time

(PD2), however, had severely reduced amounts of impregnated type I neurons (Table 3).

Nearly all impregnated type I neurons were severely affected in all except two cases: In case PD4, they were affected to different degrees, and approximately all impregnated type I neurons were normal in case PD6 (Table 5).

Discussion

Method

The variant of the Golgi technique proposed by Braitenberg et al. (1967), which was used here, is a good method for formol-fixed human autopsy material, especially if submitted to long-term storage. Spines are clearly demonstrated; dendrites are stained over long distances. The Braitenberg method is comparable to the rapid Golgi technique (Kiernan, 1981).

In general, the use of the Golgi technique in pathology involves some problems. It is difficult to differentiate between true pathological changes and artifacts. Many contradictory studies have been published on this topic (Williams et al., 1978; Buell, 1982; Fuijsawa and Nakamura, 1982; de Ruiter, 1983; Flood et al., 1987). Both the rapid-Golgi and the Golgi-Braitenberg variant seems to be sensitive to artifacts (Braitenberg et al., 1967; Buell, 1982). The Type D varicosities seen in our Parkinson cases were identical to the irregularly contoured and swollen dendrites sometimes found in control cases. Furthermore, most Golgi studies of other diseases report similar varicosities under many variable conditions (Braak et al., 1983). Thus we consider them to be unspecific. Their true pathological nature is unknown. They may be artifacts, as confirmed by Buell (1982) in rapid-Golgi-stained material; this is probably due to the long post-mortem fixation delay, as De Ruiter et al. (1983) pointed out. In our cases, the post-mortem fixation delay of at least 24 hours was relatively long. The good impregnations of nerve cells we obtained in controls indicate that the post-mortem fixation delay did not play an important role in our cases. Especially, the detection of cell residues, predominantly in Parkinson cases and only occasionally in control cases, identifies them as true pathological changes. However, their pathological versus artificial nature cannot be proven in this way. The marked difference between controls and patients with Parkinson's disease with respect to dendritic length, spine density and absence or presence of more extensive varicosities on dendritic processes confirms the reliability of the Braitenberg method.

Golgi-Cox variants are less sensitive to artifacts (Buell, 1982) but cannot be used in formalin-fixed material. Golgi-Cox methods are the technique chosen in fresh material from experimental animals. However, these variants demonstrate spines less clearly (Patt et al., 1989), though the background is much clearer than with the rapid-Golgi technique.

Neuronal types

The Golgi findings in this study are in agreement with the analysis reported by Braak and Braak (1986). Type I neurons represent neuromelanin-containing nerve cells mainly found in the pars compacta. Type II neurons do not contain neuromelanin and are mostly localized in pars reticulata. Type III neurons correspond to the small neurons described by Francois et al. (1979) and by Braak and Braak (1986).

Pathological changes in different neuronal types in cases with Parkinson's disease

Dendritic varicosities of type A-C, a severe decrease of dendritic length and a loss of dendritic spines were found solely in dopaminergic type I neurons of the pars compacta. In six out of eight Parkinson cases, nearly all of the few impregnated type I neurons were pathologically changed. The reason for the relatively well preserved type I neurons in the remaining two cases could not be clarified. Good preservation of type I neurons may be related to a short duration of the disease. Type II neurons, however, were quite normal in all Parkinson cases. These findings support the principal involvement of the nigrostriatal efferent pathway between the pars compacta and the striatum and suggest that the substantia nigra lesion is primary in Parkinson's disease. This efferent dopaminergic fibre system arises from the melanin-containing pars compacta cells (Leenders et al., 1986). The pars reticulata neurons belong to the afferent, obviously uninvolved, striatonigral pathway between the nucleus caudatus, putamen and accumbens nucleus and the pars reticulata (Leenders et al., 1986) as well as to the efferent fibre system between the pars reticulata and the thalamus (Peele, 1977).

Dendritic pathology

Lewy bodies are known to be localized in cell bodies as well as in dendrites in Parkinson's disease. Fig. 6f shows a Lewy body within the proximal portion of a primary dendrite in a nerve cell of the locus coeruleus (Palmgren stain) in a patient with Parkinson's disease. The larger type B and especially the round type C varicosities, which were absent in control cases, could be such dendritic Lewy-body inclusions. The quantity of round type C varicosities was related to the amount of Lewy bodies found in H & E sections.

The fact that type C varicosities do not seem to interfere with dendritic spine density and morphology —except in one case— suggests that these dendritic enlargements with respect to Lewy bodies do not affect dendritic morphology and thus may not have influence on function. On the other hand, the number

of varicosities exceeds the number of Lewy-body inclusions expected, and type A varicosities are too irregular in shape and too large for «only bearing Lewy bodies». Therefore the somewhat more irregular and larger varicosities are probably caused by factors other than Lewy bodys inclusions. Similarly large or irregularly contoured varicosities have been described in other degenerative diseases (Braak et al., 1983). Obviously they are not artifacts (see *Method*) and appear to be true degenerative pathological changes with unknown significance. Their occurrence in neuronal dendrites indicates that the degenerative process involves dendrites and not only cell bodies. This supports the theory that dendritic pathology is important for degeneration.

References

Bauer J. (1909). Die substantia nigra Soemmeringii. Eine vergleichende Studie nebst einem Beitrag zur Kenntnis des dunklen Pigmentes der Nervenzellen. Arb. Neurol. Inst. Wien 17, 435-512.

Braak H. (1983). Transparent Golgi impregnation: A way to examine both details of cellular processes and components of the nerve cell body. Stain Techn. 58, 91-95.

Braak H., Braak E. and Goebel H.H. (1983). Isocortical pathology in type C Niemann-Pick disease. A combined Golgi-pigmentoarchitectonic study. J. Neuropathol. Exp. Neurol. 42, 671-679.

Braak H. and Braak E. (1986). Nuclear configuration and neuronal types of the nucleus niger in the brain of the human adult. Human Neurobiol. 5, 71-82.

Braitenberg V., Guglielmotti V. and Sada E. (1967). Correlation of crystal growth with the staining of axons by the Golgi procedure. Stain Technol. 42, 227-283.

Buell S.J. (1982). Golgi-Cox and rapid-Golgi methods as applied to autopsied human brain tissue: widely disparate results. J. Neuropath. Exp. Neurol. 41, 500-507.

Danner H. and Pfister C. (1982). Sieben Neuronentypen in der Substantia nigra der Ratte. Eine Golgi-rapid-Imprägnations-studie. J. Hirnforsch. 23, 553-566.

De Ruiter J.P. (1983). The influence of post-mortem fixation delay on the reliability of the Golgi silver impregnation. Brain Res. 266, 143-147.

Flood D., Buell S.J., Horwitz G.J. and Coleman P.D. (1987). Dendritic extent in human dendate gyrus granule cells in normal aging and senile dementia. Brain Res. 402, 205-216.

Francois C., Percheron G., Yelnik J. and Heyner S. (1979). Demonstration of the existence of small local circuit neurons in the Golgi-stained primate substantia nigra. Brain Res. 172, 160-164.

Fuijsawa K. and Nakamura A. (1982). The human Purkinje cells. A Golgi study in pathology. Acta Neuropathol. (Berl.) 56, 255-264.

Hassler R. (1938). Zur Pathologie der Paralysis agitans und des postencephalitischen Parkinsonismus. J. Psych. Neurol. 48, 387-476.

Kiernan J.A. (1981). Histological and histochemical methods: Theory and practice, 1st edn. Pergamon Press, Oxford pp 262-263.

Leenders K.L., Palmer A.J., Quinn N., Clark J.C., Firnau G., Garnett E.S., Nahmias C., Jones T. and Marsden C.D. (1986). Brain dopamine metabolism in patients with Parkinson's disease measured with positron emission tomography. J. Neurol. Neurosurg. Psychiatr. 49, 853-860.

Olszewski J., Baxter D. (1954). Cytoarchitecture of the human brain stem. Karger, Basel, New York.

Patt S., Cervós-Navarro J., Stoltenburg-Didinger G. and Schreiner C. (1989). The effects of CDP-choline on newborn rat pups with experimental alcohol fetopathy. A Golgi study. Histol. Histopath. 4, 429-434.

Peele T.L. (1977). The neuroanatomic basis for clinical neurology, 3rd edn. McGraw-Hill Book Company, a Blakiston publications. New York. pp 404.

Rinvic E. and Grofova J. (1970). Observations on the fine structure of the substantia nigra in the cat. Exp. Brain Res. 11, 229-248.

Scheibel M.E., Lindsay D.R., Tomiyasu U. and Scheibel A.B. (1975). Progressive dendritic changes in aging human cortex. Exp. Neurol. 47, 392-403.

Williams R.S., Ferrante R.J. and Cavines V.S. jr. (1978). The Golgi rapid method in clinical neuropathology. The morphologic consequences of suboptimal fixation. J. Neuropathol. Exp. Neurol. 37, 13-33.

Accepted February 2, 1991