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Nigral degeneration in acquired immune deficiency syndrome (AIDS)*

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Summary. Using stereological techniques we have estimated the volume density of melanin and counted the number of pigmented and non-pigmented neuronal cell bodies in the pars compacta of the substantia nigra of 12 autopsied patients with acquired immune deficiency syndrome (AIDS) who did not have inflammation or necrosis of the midbrain or clinical parkinsonism. The total number of neuronal cell bodies was 25 % lower in AIDS ($P < 0.01$) than in 12 age-matched controls, although the volume density of neuronal melanin did not differ from that of controls because the percentage of pigmented cell bodies was higher ($P < 0.01$) and the cell bodies were more fully packed with melanin in AIDS. Also, the expected increase with age of the volume density of neuronal melanin ($P < 0.02$) and the percentage of pigmented neurons ($P < 0.01$) occurred in the controls but not in AIDS patients. Importantly, our histopathological examination showed unequivocal nigral degeneration with neuronal loss, small neuronal cell bodies packed with melanin, reactive astrocytosis and extra-cellular melanin in the AIDS patients but not in controls. Our study shows that a subclinical nigral degeneration is common in AIDS and could possibly explain the heightened suspectibility of some patients to drug-induced parkinsonism.

Key words: AIDS – Substantia nigra – Histopathology

To find out if a subclinical nigral degeneration can possibly explain the undue susceptibility of some patients with acquired immune deficiency synsrome (AIDS) to drug-induced parkinsonism [5], we studied the neuropathological and clinical findings of a selected group of autopsied AIDS patients. In this report, we present our histopathological and quantitative histological findings in the pars compacta of the substantia nigra and the other neuropathological and clinical findings of 12 of our autopsied AIDS patients.

Materials and methods

Twelve autopsied patients with AIDS and 12 control patients, of comparable ages with no neurological illnesses, comprised the study. The AIDS patients were selected to include only those without inflammatory or necrotizing lesions in the substantia nigra and those without diffuse lesions of the striatum. Table 1 lists the clinical findings of the AIDS and control patients. Six including two women and the only Haitian in the group were intravenous drug addicts. The others were homosexuals. None had parkinsonism, other abnormal movements or dementia, although one complained of poor memory 2 weeks before his death. The rest, including four who had hypotension, anemia and hypoglycemia several days before death, had no cognitive, behavioral or motor abnormalities. Two were treated with zidovudine (azidothymidine or AZT) 4 and 1 month before their deaths. One who developed generalized seizures 7 months before death was treated with diphenylhydantoin. None had been treated with neuroleptics. The ages of the AIDS patients ranged from 26 to 55 years and had a median of 29 with mean ± SE of 33 ± 2.5 years and did not differ from the controls who had a range of 22 to 49, median of 39, mean of 38 ± 2.7 years. Seven of the 12 controls were men. None were homosexuals or intravenous drug addicts. No one had any neurological symptoms and all had a normal examination. Neuropathological examination of their brains showed no abnormal gross and histopathological findings.

One to three transverse sections of the caudal midbrain at the level of the inferior colliculus were removed from the formalin-fixed brain, embedded in paraffin, cut at 5 μm and stained with hematoxylin and eosin. The section of the substantia nigra at the level of the fourth nerve nucleus was systematically divided into fields by drawing five to seven lines on the coverslip of the slide across the long axis of the left and right side of the nucleus. Next, a grid of 100 squares in the eyepiece of a microscope was placed in the central column of the substantia nigra and lined up against the line demarcating the fields (Fig. 1) by moving the stage of the

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Table 1. Clinical findings in AIDS and controls

Patient no.	Age (years)	Sex/race	Risk factor/ cause of death	Dementia	Seizures	Extrapyramidal signs	Remarks
AIDS patients							
1.	26	M/H	IV drug addict	–	–	–	–
2.	27	F/W	IV drug addict	–	–	–	–
3.	30	F/B	IV drug addict	–	–	–	–
4.	32	M/S	IV drug addict	–	–	–	Low hemoglobin Sickle cell trait
5.	33	M/B	IV drug addict	–	–	–	–
6.	55	M/B	IV drug addict	–	–	–	–
7.	26	M/B	Homosexual	–	–	–	Hypotension
8.	27	M/B	Homosexual	Memory loss	–	–	–
9.	27	M/B	Homosexual	–	–	–	–
10.	28	M/B	Homosexual	–	–	–	Hypoglycemia, zidovudine
11.	40	M/B	Homosexual	–	–	–	Hypotension
12.	41	M/B	Homosexual	–	+	–	Zidovudine
Controls							
13.	22	M/B	Myocardial infarct	–	–	–	Morbid obesity
14.	27	F/B	Breast CA	–	–	–	–
15.	28	F/B	Bronchopneumonia	–	–	–	Juvenile diabetic
16.	28	F/B	Hepatocellular CA	–	–	–	–
17.	36	M/B	Bronchogenic CA	–	–	–	–
18.	39	F/S	Breast CA	–	–	–	–
19.	45	F/W	Asthma	–	–	–	–
20.	45	F/B	Polymyositis	–	–	–	–
21.	43	M/B	Renal cell CA	–	–	–	–
22.	45	M/B	Bronchogenic CA	–	–	–	–
23.	48	F/B	Breast CA	–	–	–	–
24.	49	F/B	Breast CA	–	–	–	–

Mean age ± SE: 33 ± 2.5 years
Mean age ± SE: 38 ± 2.7 years
M: Male; F: female; B: Black; H: Haitian; S: Hispanic; W: White; IV: intravenous; CA: carcinoma; –: none

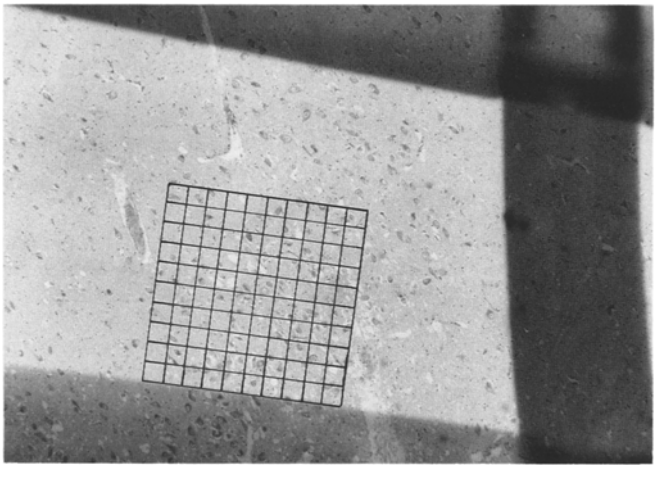

Fig. 1. Photomicrograph of the pars compacta of the substantia nigra showing the grid of 100 squares used for systematic sampling of the highest density of cell bodies with the frame of the grid lined up against the ink lines on the coverslip. The grid is not drawn to the scale of the 20X objective used for the counts where the side of the squares of the grid measured 40 μm. In this photomicrograph, the side of the squares in the grid = 55 μm

microscope. Using the 20× objective, the number of pigmented and non-pigmented neuronal cell bodies was counted within the grid including those touching the upper and right but not the left and lower sides of the frame of the grid. Also counted were the number of points (upper right hand corners of the squares in the grid) hitting neuromelanin in neuronal cell bodies. At this magnification, the side of the squares in the grid measured 40 μm. To minimize counting errors, the counting was performed by only one observer (FF) [15] without knowledge of which slides were from the AIDS or control patients.

The cell number or numerical density of profiles of neuronal cell bodies was estimated by dividing the number of neuronal cell bodies counted in the grid by the area of the grid and expressed as number per mm^2 of tissue. The percentage of pigmented neuronal cell bodies was obtained by dividing the number of pigmented neuronal cell bodies by the sum of the number of pigmented and non-pigmented neuronal cell bodies, multiplied by 100. The volume density of melanin was estimated using a derivation of Delesse's equation which states that the volume density is equal to the point density of the histological component [16]. In this study, the point density of melanin is the number of points hitting melanin divided by the total points hitting the section. Volume density of melanin was expressed as $mm^3/100\ mm^3$ of nigral tissue.

We analyzed the data using standard statistical tests including the two sampled *t*-test and Pearson's sample product moment correlation programmed on a microcomputer.

Results

Neuropathological findings

Neuropathological findings in our patients with AIDS are listed in Table 2. Apart from the concretions in the basal ganglia in one case, there were no striking gross neuropathological findings. Three had mild cerebral cortical atrophy, one slight ventricular dilatation and another mild cerebral cortical atrophy, one slight ventricular dilatation and another mild cerebral edema. Microscopic findings included: (1) a few microglial nodules without multinucleated giant cells scattered in the cerebral cortex and lower brain stem in three cases, similar to the non-specific encephalitis with microglial nodules that Petito and co-workers [14] found in 26 of 153 [17%) patients with AIDS; (2) mild arteriolosclerosis of the deep frontal white matter in two cases; and (3) and old infarction of the putamen (one case), recent necrosis of cerebellar cortex (one case), astrocytosis of the frontal cortex (one case), hippocampal sclerosis (one case) and siderocalcific vascular changes in the basal ganglia and thalami (one case). Although not necessarily abnormal [13], vascular calcification of the basal ganglia has been reported in 10 of 89 patients with AIDS [1]. The striatum was normal in all patients except one who had an old infarction of the lateral putamen and possibly another with siderocalcific vascular changes.

Nigral histopathological findings

Histopathological findings of the pars compacta of the substantia nigra in AIDS are shown in Table 3. All patients had reactive astrocytosis, 11 had smaller, more heavily pigmented neuronal cell bodies than controls

Table 2. Neuropatholgical findings in AIDS

Patient no.	Age/risk	Gross	Microscopic	Striatum	Diagnosis
1.	26/I	Cerebral edema	–	–	Cerebral edema
2.	27/I	–	–	–	No diagnostic abnormalities
3.	30/I	Mild ventricular dilatation	Hoppocampal sclerosis	–	Hippocampal sclerosis
4.	32/I	Mild atrophy	–	–	Cerebral atrophy
5.	33/I	–	Astrocytosis, frontal cortex	–	Astrocytosis, frontal
6.	55/I	–	Arteriolosclerosis of the frontal white matter	–	Arteriolosclerosis
7.	26/H	–	Microglial nodules, pons frontal white matter	–	Non-specific microglial encephalitis
8.	27/H	–	Necrosis, cerebellum	–	Cerebellar necrosis
9.	27/H	–	Microglial nodules occipital cortex	–	Non-specific microglial encephalitis
10.	28/H	Mild atrophy	One microglial nodule Frontal cortex	–	Non-specific microglial encephalitis
11.	40/H	Concretions, basal ganglia	Siderocalcific vessels basal ganglia, thalami	+	Siderocalcification, basal ganglia
12.	41/H	Mild atrophy	Infarction, old, small putamen; arteriolosclerosis, deep white matter	+	Infarction, old, small

–: No pathologic abnormalities; I: Intravenous drug addict; H: homosexual

Table 3. Histopathological findings in the substantia nigra

Patient no.	Age/sex/risk	Loss	Astrocytosis	Atrophy	Pigment	Macrophage	Blood vessels
1.	26 M/IVDA	++	++	++	++	–	–
2.	27 F/IVDA	+++	+++	+++	+++	–	–
3.	30 F/IVDA	–	+*	–	–	–	Mild AS
4.	32 M/IVDA	+++	+++	+++	+++	+	–
5.	33 M/IVDA	++	++	+++	+++	–	–
6.	55 M/IVDA	++	++	+++	+++	–	–
7.	26 M/HSXL	++	+++	++	++	–	–
8.	27 M/HSXL	–	–	+	+	–	–
9.	27 M/HSXL	–	++[a]	+	+	–	–
10.	28 M/HSXL	++	+++[a]	++	++	–	–
11.	40 M/HSXL	–	+*	+	+	–	–
12.	41 M/HSXL	+	++	++	++	+	Mild AS

M: Male; F: female; –: absent; +: mild; ++: moderate; +++: severe; IVDA: intravenous drug addict; HSXL: homosexual; AS: arteriolosclerosis

[a] Reactive astrocytes include Alzheimer-type 2 astrocytes

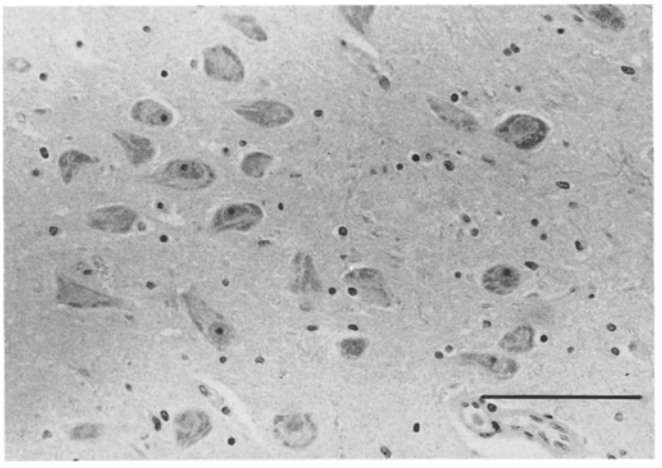

Fig. 2. Pars compacta of substantia nigra of a 27-year-old control patient who died of metastatic breast carcinoma. Bar = 100 μm

(Fig. 2, 3) and 8 had neuronal loss. Also, we found occasional perivascular pigment-laden macrophages in two cases, slight fibrous thickening of the wall of arterioles in two and Alzheimer-type 2 astrocytosis in four AIDS patients. The latter change has been reported in unspecified sites in 10 of 69 (11 %) of autopsied AIDS [1]. None the controls showed any of the above histopathological findings.

Quantitative histological estimates

Table 4 shows our quantitative histological estimates in AIDS and controls. The number of neuronal cell bodies was lower in AIDS by 25 % ($P < 0.01$) but the volume density of neuronal melanin did not differ in the two groups. We explained the latter by the higher percentage

Table 4. Quantitative histological estimates in AIDS and controls

Patient no. AIDS (n = 12)	Age/sex/race (yr)	Neuronal cell bodies Cell density (no./mm^2)	Grid/total (no.)	%Pigmented (no./100 cells)	Melanin volume density (mm^3/100 mm^3)
Intravenous drug addicts					
1.	26 M/H	55.0	25/220	97.7	2.4
2.	27 F/W	48.1	23/177	100.0	2.5
3.	30 F/B	100.0	12/192	98.0	4.0
4.	32 M/S	48.1	36/277	100.0	2.2
5.	33 M/B	35.6	32/182	100.0	1.8
6.	55 M/B	39.3	31/195	100.0	2.2
	Mean ± SE	54.4 ± 9.6		99.3 ± 0.5	2.5 ± 0.3
Homosexuals					
7.	26 M/B	57.5	15/138	98.0	2.7
8.	27 M/B	64.3	31/319	100.0	3.5
9.	27 M/B	70.6	17/192	100.0	4.0
10.	28 M/B	42.8	26/178	100.0	2.4
11.	40 M/B	56.2	33/297	100.0	2.3
12.	41 M/B	59.3	31/294	100.0	4.0
Mean ± SE		58.4 ± 3.8		99.7 ± 0.3	3.2 ± 0.3
Mean ± SE		58.3 ± 4.9*		99.4 ± 0.3*	2.8 ± 0.2
Controls (n = 12)					
13.	22/M/B	73.8	10/118	74.5	1.6
14.	27/F/B	74.5	23/274	66.6	1.5
15.	28/F/B	68.7	16/176	84.4	2.0
16.	28/F/B	81.3	24/312	87.1	1.6
17.	36/M/B	68.8	16/176	91.2	2.7
18.	39/F/S	62.5	10/100	97.1	2.1
19.	45/F/W	62.5	16/160	97.8	2.3
20.	45/F/B	83.7	13/174	96.6	3.8
21.	43/M/B	101.8	28/456	96.9	4.5
22.	45/M/B	91.2	27/394	95.8	2.2
23.	48/F/B	76.2	26/317	94.2	2.5
24.	49/F/B	102.5	15/246	99.0	3.8
Mean ± SE		78.8 ± 4.0*		90.1 ± 2.9*	2.6 ± 0.3

* $P < 0.01$ by two sampled t-test

M: Male; F: female; B: Black; H: Haitian; S: Hispanic; W: White; Neur: neuronal cell bodies

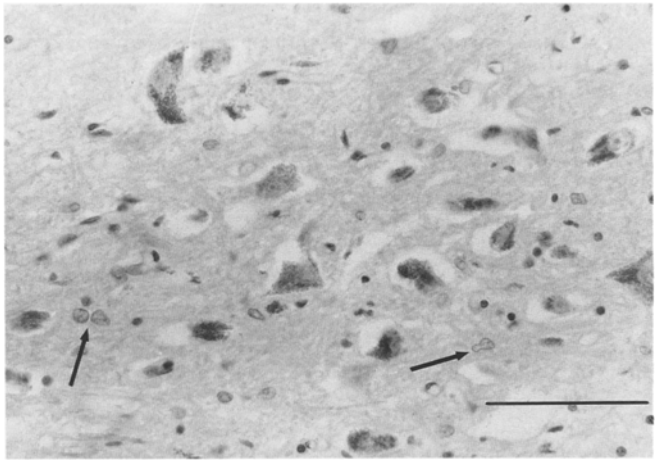

Fig. 3. Pars compacta of substantia nigra of a 27-year-old patient with AIDS showing reactive astrocytosis (*arrows*) and neuronal cell bodies that are fewer, smaller and more fully packed with neuromelanin than the control shown in Fig. 2. Bar = 100 μm

of pigmented neuronal cell bodies in AIDS ($P < 0.01$) and by the neuronal cell bodies that appear more fully packed with melanin in AIDS than controls. Also, the volume density of melanin ($r = +0.673$, $P < .02$; volume density of melanin = $-0.139 + 0.071 \times$ age) and percentage of pigmented neurons ($r = +0.844$ $P < 0.01$; percentage of pigmented neurons = $54.7 + 0.93 \times$ age) increased with the age of controls, but not in AIDS.

In the AIDS patients, those with the most severe neuronal loss, atrophy and astrocytosis had the lowest neuronal counts and also the lowest volume density of melanin.

Comparison of intravenous drug addicts and homosexuals

Although the intravenous drug addicts appeared to have more severe neuronal loss and smaller, more heavily pigmented neuronal cell bodies than the homosexuals (Table 3), the cell number and volume density of melanin did not differ statistically in the two groups (Table 4).

Discussion

Our histopathological and quantitative histopathological estimates clearly show degeneration of the pars compacta of the substantia nigra in AIDS. Although there was unequivocal loss and atrophy of neuronal cell bodies, the volume density of neuronal melanin was not significantly lower than controls because the percentage of pigmented cell bodies were higher and the cell bodies were more fully packed with melanin in AIDS than controls. Mann and Yates [10] consider heavily pigmented cells to be degenerating cells (since they only appear in patients who have no neurological illnesses after the age of 60. These same authors also found that the amount of melanin increased with age until the age of 60. Thus, our finding that the volume density of melanin and the percentage of pigmented neurons increased with the age in the controls but not in the AIDS patients further proves that the substantia nigra was abnormal in the AIDS patients.

What caused the nigral degeneration in our patients with AIDS is unclear. Since none of our patients had any inflammatory or necrotizing nigral lesions, direct infection by human immunodeficiency virus (HIV) [17] or other viruses is unlikely. However, we cannot rule out the possibility that the nigral degeneration indirectly resulted from the release of monokines from HIV-infected monocytes [6] or from viral antigens, such as gp120, that can damage neurons by increasing intracellular free calcium [4] or inhibit neuronal growth factors [9]. A nigrotoxin like the synthetic compound 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) used by intravenous drug addicts can cause nigral degeneration [2] but we have no proof that any of our patients had used MPTP. More importantly, MPTP cannot readily explain nigral degeneration in our homosexual AIDS patients. Zidovudine-treated patients can show clinical signs of neurotoxicity [3, 8] but our two patients who were given zidovudine treatment did not. Moreover, it is not known if zidovudine can cause nigral degeneration. Ischemia from localized vascular insufficiency, systemic oxygen deficiency or both is another possible cause. Four of our patients had anemia, hypoglycemia or chronic hypoxemia in the course of their illnesses, two others had arteriolosclerosis of the frontal white matter, one of whom also had an old small infarction of the putamen. One patient had a small recent cerebellar necrosis, another, focal cortical astrocytosis, and still another, hippocampal sclerosis that can best be explained by ischemia. Yet, none had any histological signs of diffuse anoxic encephalopathy or nigral infarctions. It is unlikely, therefore, that nigral degeneration resulted from vascular insufficiency in our patients. Nonetheless, our findings agree with Mizusawa and co-workers' [11] finding of a high frequency of cerebrovascular lesions in AIDS.

Since we excluded patients with gross or diffuse lesions, we doubt that our patients have retrograde nigral degeneration from destruction of their striatum or internal capsule. Whether or not transsynaptic degeneration by way of the reticular nigral neurons from cortical or subcortical lesions or both can explain the degeneration of the pars compacta of the substantia nigra in our patients remains speculative.

It is well known that nigral degeneration can be asymptomatic [7] or subclinical, and it has been well documented that structural lesions of the brain can be found in patients with AIDS who do not have neurological symptoms [1]. Thus, it is not surprising that none of our patients had parkinsonism or other abnormal movements but since none had been given neuroleptics, we do not know how susceptible they were to drug-induced parkinsonism. Nonetheless, our study shows that subclinical nigral degeneration is common in AIDS, even in patients without inflammation or necrosis of the midbrain and suggests a possible explanation for the

heightened susceptibility to drug-induced parkinsonism [5] or possibly even the parkinsonism [12] of some patients with AIDS.

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