Neuronal loss in the hippocampus in Huntington's disease: a comparison with HIV infection

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Abstract

The hippocampus is usually affected in primary dementias and the pathological changes may be severe. Knowledge of hippocampal pathology in HIV infection and Huntington's disease (HD), however, is extremely limited. A stereological technique (the optical "disector") has been used to assess neuronal populations in four areas of the hippocampus in 11 patients with HIV infection and in nine patients with HD. The HIV patients died without opportunistic infections or neoplasms affecting the brain; they had HIV encephalitis or minimal changes. The HD cases were all clinically diagnosed, had a positive family history and showed the characteristic lesions in the caudate nucleus. The neuronal counts were compared with those in nine controls. In the granule cell layer of the dentate, CA3 and CA4, there was no significant difference in the neuronal numerical density between the three groups. A striking difference between the HIV and HD groups was seen in the CA1 region. The neuronal numerical density in the CA1 area was significantly lower in the HD patients than in either the HIV patients or the controls (mean (SD) 37.5 (5.0); 70.1 (13.4); 57.9 (15.4) $\times 10^3$ per mm³, $p < 0.001$ (Students' t test)). This difference represents a neuronal loss of 35%. In all four hippocampal areas the neuronal density was higher in the HIV group than in the controls but the differences were not significant and can be explained by the higher average age of the control group. These findings contribute to the understanding of the mechanism of dementia in both AIDS and in Huntington's disease.

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disturbance.¹⁶ The cause of this dementia has not been established. However, in many of those with HIV associated dementia, encephalitis is found at necropsy and one study has shown the degree of dementia to be directly associated with the severity of inflammation.⁷

The role of this encephalitic process, which affects about 40% of patients,⁸ in the development of dementia is uncertain. A recent study has shown that there is a neuronal loss of up to 38% in the superior frontal gyrus of a group of HIV-infected patients, all but one of whom died from AIDS.⁹ Moreover, this neuronal loss occurred in the same proportions whether HIV encephalitis was present or not. These findings raised the possibility of a dual pathogenetic mechanism of HIV infection in the brain in that neuronal loss and HIV encephalitis may represent two independent processes of damage, one a toxic effect and the other an inflammatory response. In addition, the finding of neuronal loss from the frontal cortex does not support the view that HIV associated dementia is subcortical.

Similar cortical neuronal loss has been reported in the frontal cortex in Huntington's disease (HD).^{10,11} While the abnormal movements in HD have been ascribed to the anatomical and neurochemical abnormalities in the neostriatum, the underlying basis for the neuropsychiatric symptoms and dementia remains unknown.¹²

Neuropathologically, the dementia associated with HIV or with HD can each be classified as a primary dementia¹³ as structural alterations in the brain, notably cell loss from the frontal cortex, have been reported in both.⁹⁻¹¹

The early clinical presentation of dementia associated with HIV infection² and HD¹⁴ includes progressive cognitive impairment involving memory deficits and mental slowing. In addition, in both of these diseases the onset of dementia frequently occurs in early adult life, unlike Alzheimer's disease in which dementia presents at a much later age. HIV infection and HD also differ from Alzheimer's disease in that neither show the characteristic neuropathological lesions of Alzheimer's disease namely, senile plaques and neurofibrillary tangles. However, it is unclear whether the dementia in HIV infection and HD is solely attributable to the changes in the frontal cortex, and raises the question whether cell loss is seen in other areas of the cortex in either or both disorders.

The hippocampus is usually affected in

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Damage to the nervous system is a major consequence of infection with human immunodeficiency virus 1 (HIV-1) which results in a variety of neurological manifestations.¹² Abnormalities in the central and peripheral nervous system are found at necropsy in over 95% of patients with the acquired immunodeficiency syndrome (AIDS).³⁴ The most common neuropsychiatric disorder is HIV associated dementia,⁵ characterised by progressive cognitive impairment, personality change, and motor

primary dementias and severe pathological changes develop in both Alzheimer's disease and in Pick's disease. Our knowledge of hippocampal pathology in HIV infection and HD, however, is extremely limited.

A new, statistically robust stereological probe known as the optical "disector" allows three-dimensional estimation of cell number,¹⁵ thus enabling a more precise assessment of neuronal populations. The aim of this study is to quantitatively assess neuronal populations in four areas of the hippocampus to clarify the neuropathological substrate of dementia.

Methods

Hippocampal cell counts were carried out in three groups of subjects: HIV-infected patients ($n = 11$), HD patients ($n = 9$) and controls ($n = 9$). All the subjects were male. Eleven consecutive brains, with no evidence of HIV associated opportunistic infection or neoplasm, were selected for the study from the Medical Research Council Central AIDS Brain Tissue Bank (Department of Neuropathology, Institute of Psychiatry). The HIV infected cases comprised two subgroups. In the first subgroup HIV encephalitis was the sole neuropathological diagnosis (five cases: 1, 2, 4, 5, and 11), while the remaining six brains, forming the second subgroup, showed only minimal changes such as slight astrocytosis and perivascular cuffing by mononuclear cells. Ten of the 11 subjects had died with a clinical diagnosis of AIDS, and one (case 9) died, while still HIV positive, from cardiac valvular complications of osteogenesis imperfecta.

The nine Huntington's disease cases were selected from the brain collection in the Department of Neuropathology, Institute of Psychiatry. All subjects were clinically diagnosed as HD during life, had a positive family history, and post mortem examination of the brains showed the characteristic lesions in the caudate nucleus. The age of onset was known in seven of the cases [mean (SD) = 39.6 (16.2) years] and, excluding case 1 who committed suicide, the mean duration of the disease was 9.7 (4.5) years. The control group comprised nine patients: eight died of systemic illness, without cerebral pathology, whilst one had a pontine haemorrhage which did not affect the cerebral hemispheres. Although the cause of death in many of the subjects, in all groups, would have produced hypoxia there was no classic neuropathological evidence of anoxia (for example, pyknotic and eosinophilic neurons especially in CA1 region of the hippocampus). Similar findings have been reported in HIV encephalitis by other workers.¹⁶

Although the HIV status of the HD group and controls could not be ascertained, none had any evidence of being in an at-risk group. The mean (SD) age was 37.3 (7.6) in the HIV group, 50.3 (15.2) in the HD group and 47.2 (13.3) years in the controls, but the differences were not statistically significant. Fixed brain weights were known for 24 of the 29 subjects and there were no significant differences between the mean fixed brain weights of the three groups (table 1 shows patient details).

Blocks were taken from the hippocampus at the level of the lateral geniculate body. The tissue was embedded in paraffin wax, on a 5 day processing schedule. The resulting tissue shrinkage was about 10% of the section area, but it did not differ significantly between groups. Sections were cut at 20 μ m, stained with cresyl-violet and coded for assessment. Neuronal densities were quantified in the granule cell layer of the dentate, and in the CA1, CA3 and CA4 fields of the hippocampus. These anatomically discrete¹⁷ areas were delineated in accord with an earlier study.¹⁸ Quantitation was carried out using a stereological technique known as the optical "disector".¹⁹

Results

The neuronal numerical density in the four areas of the hippocampus for each subject are shown separately in tables 2–5, and group means are summarised in table 6. The mean neuronal numerical density for the control group is similar to reported values in a previous study that estimated neuronal numbers in the hippocampus.¹⁸

Analysis of variance was carried out on the data for each of the subject groups for each area quantified and this shows a striking difference between the HIV infected subjects and those with Huntington's disease. In the HIV group there is no significant difference in the neuronal numerical density in any of the

Table 1 Patient details

Case no	Age (y)	Sex	Cause of death	Brain weight (g)
Controls				
C1	35	M	Bronchopneumonia, PKU	1253
C2	44	M	Pontine Haemorrhage	1473
C3	47	M	Gastro-intestinal haemorrhage	1375
C4	51	M	Chronic cardiomyopathy	1367
C5	60	M	Myocardial infarction	1187
C6	20	M	Suicide	—
C7	57	M	Myocardial infarction	1248
C8	48	M	Ruptured aortic aneurism	1312
C9	63	M	Not specified	1345
Mean (SD)	47.2 (13.3)			1320 (90)
HIV Infected				
H1	47	M	Bronchopneumonia	1540
H2	48	M	Encephalopathy	1355
H3	22	M	Pneumocystis pneumonia	1531
H4	42	M	MAI pneumonia	1388
H5	34	M	Not specified	—
H6	37	M	Mitral valve disease	—
H7	30	M	Not specified	1540
H8	30	M	Pneumocystis pneumonia	1315
H9	32	M	Bronchopneumonia	1450
H10	38	M	Pneumocystis pneumonia	1530
H11	38	M	Pneumocystis pneumonia	—
Mean (SD)	37.3 (7.6)			1445 (94)
Huntington's Disease				
HD1	45	M	Suicide (hanging)	1594
HD2	53	M	Aspiration pneumonia	—
HD3	71	M	Cerebral thrombosis	1399
HD4	50	M	Died in fire	1520
HD5	46	M	Coronary insufficiency	1293
HD6	67	M	Bronchopneumonia	1367
HD7	31	M	Bilateral adrenal haemorrhage	1155
HD8	63	M	Myocardial degeneration	1285
HD9	27	M	Pneumonia	1131
Mean (SD)	50.3 (15.2)			1343 (162)

Table 2 Neuronal numerical density ($\times 10^3$ per mm^3) dentate.

Control group		HIV Group		HD Group	
Patient Number	Number of neurons	Patient number	Number of neurons	Patient number	Number of neurons
C1	372	H1	408	HD1	356
C2	328	H2	336	HD2	392
C3	372	H3	484	HD3	392
C4	220	H4	492	HD4	364
C5	520	H5	580	HD5	360
C6	307	H6	396	HD6	372
C7	360	H7	392	HD7	392
C8	492	H8	620	HD8	392
C9	532	H9	368	HD9	380
		H10	552		
		H11	348		
n =	9		11		9
Mean (SD)	389 (105)		452 (99)		378 (15)

Table 3 Neuronal numerical density ($\times 10^3$ per mm^3) CA1 region

Control group		HIV Group		HD Group	
Patient Number	Number of neurons	Patient number	Number of neurons	Patient number	Number of neurons
C1	48.0	H1	80.0	HD1	33.3
C2	78.7	H2	57.3	HD2	44.0
C3	44.0	H3	90.7	HD3	42.7
C4	50.7	H4	61.3	HD4	40.0
C5	70.7	H5	60.0	HD5	37.3
C6	46.7	H6	72.0	HD6	32.0
C7	40.0	H7	62.7	HD7	29.3
C8	80.0	H8	86.7	HD8	38.7
C9	62.7	H9	72.0	HD9	40.0
		H10	80.0		
		H11	48.0		
n =	9		11		9
Mean (SD)	57.9 (15.4)		70.1 (13.4)	37.5** (5.0)	

**Significantly different from control $t = 4.19$; $p < 0.001$ (Student's t -test).Table 4 Neuronal numerical density ($\times 10^3$ per mm^3) CA3 region

Control group		HIV Group		HD Group	
Patient Number	Number of neurons	Patient number	Number of neurons	Patient number	Number of neurons
C1	49.3	H1	45.3	HD1	45.3
C2	50.7	H2	41.3	HD2	37.3
C3	44.0	H3	57.3	HD3	41.3
C4	41.3	H4	50.7	HD4	45.3
C5	38.7	H5	41.3	HD5	38.7
C6	42.7	H6	50.6	HD6	44.0
C7	41.3	H7	44.0	HD7	40.0
C8	48.0	H8	52.0	HD8	40.0
C9	33.3	H9	48.0	HD9	42.7
		H10	45.3		
		H11	40.0		
n =	9		11		9
Mean (SD)	43.3 (5.5)		46.9 (5.4)		41.6 (2.9)

Table 5 Neuronal numerical density ($\times 10^3$ per mm^3) CA4 region.

Control group		HIV Group		HD Group	
Patient Number	Number of neurons	Patient number	Number of neurons	Patient number	Number of neurons
C1	44.0	H1	52.0	HD1	41.3
C2	34.7	H2	46.7	HD2	34.7
C3	36.0	H3	56.0	HD3	34.7
C4	36.0	H4	44.0	HD4	34.7
C5	34.7	H5	36.0	HD5	40.0
C6	37.3	H6	37.3	HD6	34.7
C7	40.0	H7	34.7	HD7	38.7
C8	38.7	H8	30.7	HD8	37.3
C9	38.7	H9	34.7	HD9	40.0
		H10	32.0		
		H11	37.3		
n =	9		11		9
Mean (SD)	37.8 (3.0)		40.1 (8.4)		37.3 (2.7)

four areas quantified compared with that in the control group, although the mean neuronal density in all areas is consistently higher than in the controls.

The striking and significant difference between the groups is seen in one region of the hippocampus. In the HD group the neuronal density in the CA1 region ($37.5 (5.0) \times 10^3$ per mm^3) is significantly less ($p < 0.001$; Student's t test) than that observed in either the control ($57.9 (15.4) \times 10^3$ per mm^3) or HIV group ($70.1 (13.4) \times 10^3$ per mm^3). This difference represents a neuronal loss of some 35%. In the other regions quantified, the neuronal density in the HD group is not significantly different from the control or HIV group, although the mean values are consistently less than those in the controls.

An analysis of variance was carried out on the data for the two HIV subgroups and the control group. This showed that neither subgroup was significantly different from controls nor was there any significant difference between the subgroups in respect of any of the hippocampal regions quantified.

Discussion

This is the first quantitative study to have revealed cell loss of 35% in the hippocampus in HD and shows the changes to be restricted to area CA1. Little attention has been paid to hippocampal changes in HD, since an early study²⁰ reported cell density to be normal. However, a more recent morphometric study of macroscopic sections from 30 cases of HD has reported a reduction of hippocampal area of some 20% compared with controls,¹² and cited the early study²⁰ as evidence of normal cell density. The authors explain this apparent discrepancy between reduction of area and normal neuronal density as being a result of a combination of neuronal loss and a proportionate tissue shrinkage. Moreover, one of the authors has stated elsewhere "to have been more impressed by the normality of the hippocampus than by any abnormality",²¹ and commented that the use of sophisticated techniques may reveal changes in the hippocampus not yet detected. The present study which has used a recently developed technique for assessing neuronal density would support this statement since it has detected a reduction in neuronal density in one area of the hippocampus. In addition, since CA1 accounts for a substantial proportion of the total hippocampal volume,¹⁸ a 35% reduction of neuronal density in CA1 could account in part for the 20% reduction in hippocampal area previously reported.¹²

In our study there are insufficient details to assess clinically the degree of dementia in the HD and HIV infected patients but the neuronal loss in HD is a potential cause of dementia. Huntington's disease,²² and more recently, HIV associated dementia,² have both been clinically labelled as subcortical dementias. However, pathologically, the earlier studies⁹⁻¹¹ showing neuronal loss in the frontal cortex in both conditions, together

Table 6 Mean (SD) neuronal numerical density in four areas of the hippocampus in control, HIV and HD groups.

Patient group	Cell density ($\times 10^{-3} \text{ mm}^{-3}$)			
	Dentate	CA1	CA3	CA4
Controls (n = 9) Mean (SD)	389 (105)	57.9 (15.4)	43.3 (5.5)	37.8 (3.0)
HIV Infected (n = 11) Mean (SD)	452 (99)	70.1 (13.4)	46.9 (5.4)	40.1 (8.4)
Huntington's disease (n = 9) Mean (SD)	378 (15)	37.5** (5.0)	41.6 (2.9)	37.3 (2.7)

**Significantly different from control $t = 4.19$; $p < 0.001$ (Student's t test).

with the present study showing neuronal loss in the hippocampus appear to raise some doubt that the dementias are solely subcortical, since all show that there is cortical pathology in addition to the subcortical components.

The selective vulnerability of hippocampal regions has not yet been fully explained, but the CA1 field is more vulnerable to damage than other areas. For example CA1 is preferentially affected in a variety of conditions such as hypoxia,²³ Alzheimer's disease,²⁴ and Pick's disease.²⁵ Thus the present findings in HD would confirm the selective vulnerability of this area.

The underlying mechanisms of selective neuronal damage in HD remains uncertain, although it has been proposed that an excitatory neurotransmitter, glutamate,²⁶ and its analogues such as quinolinic acid²⁷ may act as neurotoxins. In vitro studies using rat hippocampal cell cultures²⁶ have shown dentate granule cells to be relatively resistant to glutamate-induced neurodegeneration, while cells from CA1 and CA3 were significantly more vulnerable. It was further shown that the vulnerable, but not the resistant, neurons expressed glutamate receptors which mediated large rises in intracellular calcium and subsequent degeneration. In HD a reduction of glutamate receptors on striatal neurons has been reported²⁸ which would suggest that glutamate metabolism may have an important role in the pathogenesis of the cell loss in CA1 seen in our study.

Although there is no significant difference in the neuronal numerical density, in any of the regions examined, between the HIV group and controls the values are consistently higher in the former group. This may be a reflection of the difference in the mean ages of the two groups. Although this difference is not significant, the mean for the HIV group is some 10 years less than the controls, and neurons decrease by some 3.6% per decade in the CA1 region²⁹ of the hippocampus.

The absence of hippocampal neuronal loss in the HIV group is interesting as it may provide an indication that the mechanism of cell damage in AIDS is different from that in HD, or may simply reflect a difference in duration of the disease processes. Although the duration of the symptoms is unknown in the HIV group, it is likely to be considerably shorter than the mean duration of 9.7 years in the HD group. Thus it is possible that the

patients with HD died at an advanced stage of dementia by which time the hippocampus was involved, whereas the HIV patients died of other complications at an earlier stage of the disease before hippocampal involvement. Our study was retrospective and there is a paucity of information regarding the cognitive status of the patients. It is hoped, however, that a prospective study, being carried out at present, will address this problem.

These findings contribute to the understanding of the mechanism of dementia in both AIDS patients and in Huntington's disease and further delineate and quantify the extent of the neuropathological lesions in both conditions. It is anticipated that a further comparative study of selective neuronal loss in HIV infection and HD will lead to a better understanding of these diseases.

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