Short report

Dopaminergic neurotransmitter systems in Alzheimer's disease and in Down's syndrome at middle age

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SUMMARY In 15 patients with Alzheimer's disease and in 10 with Down's syndrome at middle age, there was severe atrophy, neurofibrillary degeneration and loss of pigmented dopaminergic nerve cells from ventral tegmental area (A10) whereas nerve cells in neighbouring substantia nigra (A9) were much less affected in all three respects. It is suggested that these findings may represent different patterns of damage within the two systems in these conditions which may relate to the presence of Alzheimer type changes (senile plaques) within their respective projection fields.

While an atrophy, neurofibrillary degeneration and loss of those nerve cells comprising the cortically projecting cholinergic,¹⁻¹⁵ noradrenergic^{11 14 16-21} and serotonergic^{11 21-23} neurotransmitter systems is well established in Alzheimer's disease, less is definitely known concerning the dopaminergic systems based on the melanin pigmented neurons of the substantia nigra (SN), (A9) and ventral tegmental area (VTA), (A10). Biochemical studies²⁴⁻²⁷ indicate that dopamine and/or homovanillic acid (HVA) concentrations are not greatly altered within caudate nucleus and putamen,²⁴⁻²⁷ though losses of both these substances have been recorded within frontal cortex and amygdala.^{25 27} These findings may represent different patterns of damage within SN and VTA nerve cell groups which have differing projection systems, the SN projecting mainly to basal ganglia via the nigrostriatal tract and VTA to cerebral cortex (amygdala and frontal cortex) via mesolimbic and mesocortical pathways. In this study we have examined neurons of SN and VTA in Alzheimer's disease and in middle aged patients with Down's syndrome for Alzheimer type changes (neurofibrillary tangles)

and we have also assessed the degree of damage to these systems in both conditions by counting the numbers of nerve cells and by measuring the volume of their nucleolus.

Materials and methods

Brains were obtained at necropsy from 15 moderately to severely demented patients of age range 53-89 years (mean 73.3 ± 3.1 (SE) years) dying with histologically verified Alzheimer's disease, and from 10 middle aged patients with Down's syndrome (age range 51-65 years; mean 59.1 ± 1.4 (SE) years) whose brains also showed numerous senile plaques and neurofibrillary tangles within cerebral cortex and hippocampus. From the formalin fixed brains, a standard block of mid brain was cut at the level of the red nucleus to include SN and VTA nerve cell groups, and from these blocks paraffin sections were cut at 5 μ m and 16 μ m thickness. Sections cut at 5 μ m were stained using conventional neuropathological techniques including a modified Palmgren method²⁸ for neurofibrillary tangles, whereas those cut at 16 μ m were stained for RNA with Azure B.²⁹ In these latter sections, the mean number of nucleolated nerve cells in SN and VTA on one side only per section were counted, using their neuromelanin as a natural marker^{30 31} and the mean volume of their nucleolus was also measured.^{11 21} Overall mean values of cell counts and nucleolar volume were calculated for Alzheimer's disease and Down's syndrome groups and these were compared, using the *t* test, with values from appropriate age-matched control patients (values drawn from a series of 67 mentally able patients of age range 10-97 years (see reference 21 for substantia nigra data; unpublished data for VTA)).

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Results

In both Alzheimer's disease and Down's syndrome, the SN was generally well preserved, with only an occasional cell undergoing degenerative

depigmentation or showing neurofibrillary tangle formation (fig a and b). However, in VTA a variable picture of cell loss was seen and nerve cells containing neurofibrillary tangles were common in both Alzheimer's disease and Down's syndrome (fig c and

Table Mean ($\pm$ SEM) values of number (per section) and nucleolar volume of nerve cells of substantia nigra and ventral tegmental area in patients with Alzheimer's disease and Down's syndrome, with appropriate age-matched control values. Percentage loss in Alzheimer's disease and Down's syndrome (where significant) is given in parentheses

Patient group	Age (yr)	Substantia nigra		Ventral tegmental area	
		Cell number	Nucleolar volume	Cell number	Nucleolar volume
Alzheimer's disease (n = 15)	73.3 $\pm$ 3.1	409.4* $\pm$ 28.7 (17.3)	39.7† $\pm$ 2.2 (18.8)	16.4† $\pm$ 2.7 (43.1)	21.9‡ $\pm$ 1.7 (35.0)
Control (n = 10)	74.7 $\pm$ 3.0	494.8 $\pm$ 17.8	48.9 $\pm$ 1.7	28.8 $\pm$ 2.2	33.7 $\pm$ 1.4
Down's syndrome (n = 10)	59.1 $\pm$ 1.4	456.7 $\pm$ 44.7	37.3‡ $\pm$ 2.0 (27.3)	14.5‡ $\pm$ 2.2 (52.6)	17.7‡ $\pm$ 1.6 (50.3)
Control (n = 10)	60.0 $\pm$ 1.5	548.3 $\pm$ 15.2	51.3 $\pm$ 1.6	30.6 $\pm$ 1.9	35.6 $\pm$ 1.3
Alzheimer's disease (n = 6)	60.7 $\pm$ 2.3	461.8* $\pm$ 23.6 (15.8)	34.6* $\pm$ 8.5 (30.6)	12.0† $\pm$ 3.7 (60.8)	18.5‡ $\pm$ 1.5 (48.0)

*, †, ‡, denotes p < 0.05, < 0.01, < 0.001 versus controls, respectively.

Fig Neurofibrillary tangles in nerve cells of substantia nigra (a and b) and ventral tegmental area (c and d) in patients with Alzheimer's disease (a and c) and Down's syndrome at middle age (b and d). Palmgren $\times$ 540.

d). These changes were often more severe in Alzheimer's disease patients under 75 years of age.

Cell counting (table) showed that when compared with age-matched control patients, the number of surviving cells in SN was slightly lower (15–17%) ($p < 0.05$) in Alzheimer's disease, but not significantly lower in Down's syndrome. The number of surviving cells in VTA, however, was significantly lower (40–60%) ($p < 0.01$ at least) in both patient groups (table). Nucleolar volume was reduced in cells of SN, in both groups, by 18–30%, whereas that in cells of VTA was decreased ($p < 0.001$) by more than 35% in both groups. No significant differences in either cell number or nucleolar volume were noted in either SN or VTA between the Down's syndrome and the six youngest Alzheimer's disease patients (which matched the Down's syndrome group for age) (table).

Discussion

Findings that the dopaminergic cells of the VTA (A10) are severely affected in Alzheimer's disease and Down's syndrome at middle age, whereas those of SN (A9) are much less damaged, has important pathogenetic implications. Neurons of VTA project to frontal and limbic areas of cortex whereas those of SN project to basal ganglia. It is difficult to conceive of a pathogenic event acting directly on dopaminergic cell bodies that would severely affect those of VTA, while sparing (relatively) those of SN. What seems more likely is that the primary damage to these neurons occurs within their terminal fields, with reduction in nucleolar volume (atrophy), neurofibrillary degeneration and loss of perikarya following as secondary retrograde changes. The observations that the pathological hallmarks of Alzheimer's disease (that is, plaques and tangles) are numerous in frontal and limbic areas, but scarce in basal ganglia, would be consistent with this argument.

There is evidence from other transmitter systems to support the concept that the subcortical damage of Alzheimer's disease and Down's syndrome is secondary to primary changes within cerebral cortex. For example, Perry and colleagues² have shown that loss of cortical cholineacetyltransferase activity (CAT) far exceeds nerve cell loss within the parent nucleus basalis; indeed Pearson *et al*³² have identified patients with Alzheimer's disease who show loss of cortical CAT without decrease in cell number in nucleus basalis. The presence of CAT immunoreactivity within neurites of senile plaques³³ implies that the senile plaque may be the site of the damage to nerve terminals, as do the quantitative relationships linking cell loss in different parts of nucleus basalis^{11–14} and locus caeruleus,^{11 34} to plaque formation within their appropriate target

fields in the cerebral cortex. Observation of tyrosine-hydroxylase immunoreactivity within plaque neurites³⁵ suggests that the dopaminergic cells of VTA may also be damaged in a similar way to those of nucleus basalis and locus caeruleus. The much lesser degree of neurofibrillary degeneration, atrophy and loss of cells in SN, may relate either to the few plaques within basal ganglia, or it may arise from some degree of collateralisation (either "intended" or "aberrant") with the cerebral cortex.

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