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Striatal dopaminergic markers in dementia with Lewy bodies, Alzheimer's and Parkinson's diseases: rostrocaudal distribution

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Summary

Dementia with Lewy bodies (DLB) is a neuropsychiatric disease associated with extrapyramidal features which differ from those of Parkinson's disease, including reduced effectiveness of L-dopa and severe sensitivity reactions to neuroleptic drugs. Distinguishing Alzheimer's disease from DLB is clinically relevant in terms of prognosis and appropriate treatment. Dopaminergic activities have been investigated at coronal levels along the rostrocaudal striatal axis from a post-mortem series of 25 DLB, 14 Parkinson's disease and 17 Alzheimer's disease patients and 20 elderly controls. [^{3}H]Mazindol binding to the dopamine uptake site was significantly reduced in the caudal putamen in DLB compared with controls (57%), but not as extensively as in Parkinson's disease (75%), and was unchanged in Alzheimer's disease. Among three dopamine receptors measured (D1, D2 and D3), the most striking changes were apparent in relation to D2. In DLB, [^{3}H]raclopride binding to D2 receptors was significantly reduced in the caudal putamen (17%) compared with controls, and was significantly lower than in Parkinson's disease at all levels. D2 binding was significantly elevated at all coronal levels in Parkinson's disease compared with controls, most extensively in the rostral putamen (71%). There was no change from the normal pattern of D2 binding in Alzheimer's disease. The only significant alteration in D1 binding ([^{3}H]SCH23390) in the groups examined was an elevation (30%) in the caudal striatum in Parkinson's disease. There were no differences in D3 binding, measured using [^{3}H]7-OH-DPAT, in DLB compared with controls. A slight, significant decrease in D3 binding in the caudal striatum of Parkinson's disease (13%) patients and an increase in Alzheimer's disease (20%) in the dorsal striatum at the level of the nucleus accumbens were found. The concentration and distribution of dopamine were disrupted in both DLB and Parkinson's disease, although in the caudate nucleus the loss of dopamine in DLB was uniform whereas in Parkinson's disease the loss was greater caudally. In the caudal putamen, dopamine was reduced by 72% in DLB and by 90% in Parkinson's disease. The homovanillic acid : dopamine ratio, a metabolic index, indicated compensatory increased turnover in Parkinson's disease, which was absent in DLB despite the loss of substantia nigra neurons (49%), dopamine and uptake sites. These differences between DLB, Parkinson's disease and Alzheimer's disease may explain some characteristics of the extrapyramidal features of DLB and its limited response to L-dopa and severe neuroleptic sensitivity. The distinct changes in the rostrocaudal pattern of expression of dopaminergic parameters are relevant to the interpretation of the *in vivo* imaging and diagnosis of DLB.

Keywords: human striatum; dementia with Lewy bodies; Alzheimer's disease; Parkinson's disease; dopamine receptors

Abbreviations: ANOVA = analysis of variance; DLB = dementia with Lewy bodies; HVA = homovanillic acid; 7-OH-DPAT = *R*(+)-7-hydroxy-dipropylaminotetralin-2-*N*,*N*-di[2,3(*n*)-propylamino]-7-hydroxy-1,2,3,4-tetrahydronaphthalene; PPAP = *R*(–)-*N*-(3-propyl-1-propyl)-1-phenyl-2-aminopropane hydrochloride; SCH23390 = R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride

Table 1 *Details of subjects*

Controls

Case no.	Age (years)	Sex	PM delay (h)	Cause of death	Coronal levels at which parameters were measured				
					DAT	D1	D2	D3	DA and HVA
1	89	F	24	Pulmonary embolism	14	14	15	14	14
2	78	F	17	Cancer	10	10	10,11		10
3	56	M	18	Cancer	11	11	10		11
4	94	F	74	Renal and heart failure	10,12,13,14	10,11,13,14	10,13,15	10,11,13,14	10,12,13,14
5	80	F	48	Ischaemic heart disease	11	10	11		11
6	77	M	48	Cancer	13	13	13	13,14	14
7	72	F	14	Cancer	11	10	11		11
8	78	M	11	Bronchopneumonia				11	
9	63	M	16	Cancer + haemorrhage	12	11	11,13		12
10	82	F	15	Emphysema	12	11	11	11	12
11	78	M	42	Heart disease + pneumonia	13	14	13	14	13
12	82	M	24	Cancer	11	11	11		11
13	75	M	12	Ischaemic heart disease	11	13	11		11
14	84	M	76	Cancer	12,13	11,13	13	13	12,13
15	76	F	24	Cancer	11	11	11	11	11
16	90	F	48	Ischaemic heart disease	10,11	11	10		10,11
17	82	F	12	Bronchopneumonia	13,14	13	13,15	14	13,14
18	55	M	24	Cancer	10,11	10	10,11	10	10,11
19	91	F	48	Ischaemic heart disease	11		11	11	11
20	64	F	89	Cancer	12,13,14	13,14	13,15	13	12,13,14
Mean	77.3		34.2						
SD	10.9		23.8						

Dementia with Lewy bodies

Case no.	Age (years)	Sex	PM delay (h)	Cause of death	Disease duration (years)	Neuroleptic medication*	EPS†	Coronal levels at which parameters were measured				
								DAT	D1	D2	D3	DA and HVA
21	78	F	48	Ischaemic heart disease	1	Yes S	+	11		11	13	11
22	71	F	10	Bronchopneumonia	6	No	No	12	13	13	14	12
23	74	M	45	Duodenal ulcer haemorrhage	1	No	+	11	10	11	10	11
24	75	M	24	Bronchopneumonia	1.5	Yes	No?	11	11	11	11	11
25	78	M	47	Cancer	2	No		13	13	14	14	13
26	80	M	5	Pulmonary embolism	?	Yes S	+++	13	13	11	13	13
27	90	M	52	Bronchopneumonia	4	Yes S	+	12	13	11	13	13
28	84	M	16	?	1	Yes S	+	11	11	11	11	11
29	83	M	72	Ischaemic heart disease	11	Yes S	+	11,13	11,13	11,13	10,13	11,13
30	66	F	10	Pyelonephritis	3	Yes S	+	11,13	11,13	11,14	10,14	11,13
31	82	F	24	Bronchopneumonia	?	Yes	+	11	11	11	11	11
32	69	M	27	Ischaemic heart disease	2	No	No	11,13	11,13	11	10,14	11,13
33	85	M	46	Bronchopneumonia	?	No	+	12	13	14	13	12
34	78	M	24	?	?	Yes	+	11	11	11	10	11
35	77	F	32	Pyelonephritis	11	Yes S	+	11	11	11	11	11
36	72	M	18	Bronchopneumonia	6	Yes		11	10	11		10,11
37	82	M	90	Bronchopneumonia	1	Yes S	++	11	11	11	10	11
38	90	M	24	Bronchopneumonia	5	Yes	++	11	10	11	11,14	10
39	83	M	61	Pneumonitis	2	Yes S	++	11	10	11	10	
40	78	M	14	Bronchopneumonia	5	No		11	11	11	10	11
41	69	M	7	Cachexia	4	No		12	13	11	11	12
42	66	F	72	Bronchopneumonia	3	?	+	13	13	13		
43	85	F	18	Bronchopneumonia	5	No	+	13	13	14		13
44	84	M	51	Cancer	2	No	+	11	11	11		10,11
45	74	F	12	Heart disease	1	Yes	+	13	13	13	13	13
Mean	78.12		33.96									
SD	6.8		23.5									

Table 1 (contd)
Parkinson's disease

Case no.	Age (years)	Sex	PM delay (h)	Cause of death	Disease duration (years)	L-Dopa medication	Neuro-leptic medication	Coronal levels at which parameters were measured				
								DAT	D1	D2	D3	DA and HVA
46	73	M	22	Peritonitis	8	Yes	No	11	11	11		11
47	65	M	60	Cancer	20	Yes	No	10	10	10	10	10
48	72	F	35	Bronchopneumonia	3	Briefly	No	11	11	11	11	10
49	72	F	80	Myocardial infarction	7	Yes	No	14	13	13	11	13
50	72	M	7	Bronchopneumonia	11	Yes	No	11	13		13	11
51	65	M	30	Ischaemic heart disease	3	Yes	No				13	
52	70	M	35	Pulmonary embolism	3	No	No	10,11,12	10,11	10,11,12	10,11	10,11,13
53	86	M	48	Ischaemic heart disease	13	Yes	No	12	12	10	11	13
54	84	M	78	Pulmonary embolism	9	Yes	Yes	12	13	12	11	13
55	67	M	24	Renal failure	5	Yes	No	10	10	10	10	10
56	78	M	24	Bronchopneumonia	4	Yes	No	11,13	13	11,13	11,13	11,13
57	83	F	120	Bronchopneumonia	8	Yes	No	13	12	13	13	13
58	73	F	8	Cancer	11	Yes	Yes	10,11	11	10,11		10,11
59	83	F	48	?	?	Yes	No	13	12	13	10	13
Mean	74.5		44.21									
SD	7.1		31.4									

Alzheimer's disease

Case no.	Age (years)	Sex	PM delay (h)	Cause of death	Disease duration (years)	Neuroleptic medication	EPS†	Coronal levels at which parameters were measured				
								DAT	D1	D2	D3	DA and HVA
60	89	F	37	Bronchopneumonia	1	Yes	No	9	10	14	10	10,13
61	91	M	21	Coronary occlusion	3	No	No	11,13	11	11	11	11
62	66	F	39	Bronchopneumonia	3	Yes	Yes	9,11	11	11		10,11
63	77	M	96	Bronchopneumonia	6	Yes	No	11	10	11	10	10
64	76	F	48	?	?	No		13	13	14	13	13
65	86	F	53	Bronchopneumonia	?	No		13	13	14	13	13
66	84	F	21	Cardiac failure	?	Yes		11	11	11	11	11
67	74	M	36	Pulmonary embolism	13	Yes		11	11	11	11	11
68	83	F	24	Peritoneal abscess	5	Yes	No	13	13	14	13	13
69	89	M	28	Myocardial infarction	6	Yes, initially		11	11	11	11	11
70	84	M	24	Bronchopneumonia	7	Yes	No	13	13	14	13	13
71	83	F	24	Cancer	1	No	No	11	11	11		11
72	88	F	48	Ischaemic heart disease	10	Yes	No	11	11	11		11
73	83	F	90	Cancer, pulmonary embolism	5	Yes	Yes	11	11	11	11	11
74	69	M	24	Bronchopneumonia	9	No	Yes	9,11,13	10,11	11	10	11
75	72	M	10	Bronchopneumonia	5	Yes	Yes, terminally	9				
76	79	F	72	?	?	?		13	13	14		13
Mean	80.76		40.88									
SD	7.4		24.7									

PM = post-mortem; *S = severe reaction; †EPS = extra pyramidal symptoms: + to +++ = increasing severity of parkinsonism.

Introduction

Dementia with Lewy bodies (DLB) is a progressive, degenerative dementia which is the second commonest after Alzheimer's disease. Clinical features of DLB include disturbances of consciousness and recurrent visual hallucinations, with progressive cognitive decline which develops to severe dementia. Spontaneous extrapyramidal symptoms occur in the majority of cases, including masked face, stooped posture, slow gait and rigidity. Resting tremor also occurs, but probably less than in Parkinson's disease (Galasko *et al.*, 1996; McKeith *et al.*, 1996). These parkinsonian signs are associated with reduced concentration of striatal dopamine (Langlais *et al.*, 1993; Marshall *et al.*, 1994; Piggott and Marshall, 1996) and reduced neuron density in the substantia nigra pars compacta (Perry *et al.*, 1990,

Table 2 *Pathological findings in DLB, Parkinson's disease and Alzheimer's disease*

Group	Lewy bodies		Substantia nigra neuron numbers		Plaque count per mm^2		NFT count per mm^2		Braak stage	
	SN	Cortex	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Control	–	–	494 ± 142	307–683	3.4 ± 4.7	0–14.1	0.04 ± 0.12	0–0.5	2.3 ± 1.3	0–4
DLB	+	++	251 ± 119	79–515	12.9 ± 7.9	0–26	1.2 ± 2.6	0–9	3.3 ± 1.4	0–5
PD	+	+	156 ± 82	49–356	4.1 ± 6.5	0–22	0.15 ± 0.2	0–0.6	2.2 ± 1.4	0–4
AD	–	–	489 ± 197	241–948	23.5 ± 18.1	7.9–47	12.9 ± 5.8	1.6–23.2	5.3 ± 0.7	4–6

AD = Alzheimer's disease; PD = Parkinson's disease; NFT = neurofibrillary tangles; SN = substantia nigra.

1993). Dopamine uptake site density in the striatum is also reduced but to a lesser extent than in Parkinson's disease (Piggott *et al.*, 1998), and D2 receptors are not upregulated (Piggott *et al.*, 1994, 1998). Extrapyramidal signs in DLB can be severely exacerbated, or appear for the first time, following administration of the neuroleptic drugs that are usually prescribed to control psychotic symptoms such as hallucinations and delusions. Severe neuroleptic reactions, which do not necessarily resolve upon drug withdrawal, include rigidity, reduced consciousness, pyrexia, falling, postural hypotension and collapse (McKeith *et al.*, 1992, 1995; Ballard *et al.*, 1998*a*).

In Parkinson's disease, a progressive extrapyramidal movement disorder (rigidity, bradykinesia and tremor) is accompanied by increasing loss of striatal dopamine and severely reduced substantia nigra neuron density as well as reduced dopamine uptake sites, particularly in the putamen (Antonini *et al.*, 1995; Wilson *et al.*, 1996). At early stages of the disease or without dopamine replacement therapy, increased dopamine D2 receptors have been demonstrated in the striatum, both *in vitro* (Guttman, 1987; Piggott and Marshall, 1996) and by *in vivo* imaging (Antonini *et al.*, 1995, 1997*b*).

In Alzheimer's disease, extrapyramidal features may also emerge, particularly in the later stages (Stern *et al.*, 1996; Lopez *et al.*, 1997). Reports of nigrostriatal neurochemical abnormalities vary, with some reports of little or no loss in dopamine uptake *in vivo* in Alzheimer's disease (Tyrrell *et al.*, 1990; Donnemiller *et al.*, 1997) and other reports of reduced dopamine uptake sites *in vitro* (Sahlberg *et al.*, 1998) and *in vivo* (Rinne *et al.*, 1998). There are reports that substantia nigra neuron numbers and striatal dopamine concentration are unchanged (Perry *et al.*, 1990, 1993; Love *et al.*, 1996; Liu *et al.*, 1997), but there is one report of reduced substantia nigra neuron density (Kazee *et al.*, 1995). Extrapyramidal symptoms in Alzheimer's disease may be associated with neurofibrillary tangles in the substantia nigra (Liu *et al.*, 1997). Unaltered or reduced D2 receptors have also been reported (Pizzolato *et al.*, 1996). This variability of reported findings of dopaminergic activities in Alzheimer's disease may be due to heterogeneity within the cases selected and the symptoms displayed (Forstl *et al.*, 1994; Victoroff *et al.*, 1996) and also to the potential inclusion of patients with DLB (Ellis *et al.*, 1996; Kalra *et al.*, 1996).

We have recently reported increasing gradients of dopamine uptake sites and D2 receptors in the striatum of normal elderly individuals from the head of the caudate rostrally to the internal globus pallidus caudally (Piggott *et al.*, 1999). The number of D1 receptors was higher in the rostral putamen, D3 receptors were mainly expressed in the ventral striatum, and dopamine and homovanillic acid concentrations were higher at the level of the nucleus accumbens and caudal to the anterior commissure (Piggott *et al.*, 1999).

In the present paper we report the rostrocaudal striatal distribution of dopaminergic markers in a post-mortem series of DLB, Parkinson's disease and Alzheimer's disease cases defined clinically and pathologically. We have distinguished changes characteristic of disease-related movement disorder from normal, aged individuals, and established parameters which have the potential to differentiate DLB from Alzheimer's disease and Parkinson's disease by *in vivo* imaging.

Methods

Cases

The series, selected from the Newcastle Brain Tissue Bank, included 25 cases with DLB, 14 Parkinson's disease cases and 17 Alzheimer's disease cases. Controls were 20 normal elderly individuals with no history of neurological or psychiatric disease, no record of L-dopa or neuroleptic prescription and no evidence of significant age-related neurodegeneration. Permission for post-mortem and donation of brain tissue were obtained by prior consent from next of kin, in accordance with the rules of the Joint Ethics Committee, Newcastle and North Tyneside Health Authorities, and brain-banking procedures were in line with MRC guidelines. Brains were removed at autopsy. The right hemisphere and brainstem were fixed in formalin and examined histologically, while the left hemisphere was coronally sliced, snap-frozen and stored at –70°C. Table 1 shows demographic case variables and the coronal levels that were quantified for each parameter. Clinically, DLB cases fulfilled the criteria of McKeith and colleagues (McKeith *et al.*, 1992, 1996) and Alzheimer's disease cases were diagnosed with regard to published criteria (McKhann *et al.*, 1984). All Parkinson's disease cases presented with clinical signs of extrapyramidal movement

disorder, including tremor, rigidity and akinesia (Perry *et al.*, 1985). The presence of dementia in Parkinson's disease was assessed using previously described criteria (Perry *et al.*, 1990); if dementia developed within a year of onset of motor symptoms the cases were assigned to the DLB group (McKeith *et al.*, 1996). Pathologically, DLB and Parkinson's disease were distinguished from Alzheimer's disease by the presence of brainstem Lewy bodies, cortical Lewy bodies, Lewy neurites in the CA2/3/4 segments of the hippocampus, and low or moderate Alzheimer-type pathology with fewer tangles than found in Alzheimer's disease (Perry *et al.*, 1990, 1996) (Table 2). Parkinson's disease cases showed greater substantia nigra neuron loss, less extensive Lewy body formation in the neocortex and less Alzheimer-type pathology than DLB cases. Pathologically, Alzheimer's disease cases showed tangles and neuritic plaques in the hippocampus and neocortex, which were present at sufficient densities to satisfy published criteria (Braak and Braak, 1991; Mirra *et al.*, 1991) (Table 2).

Neuropathological methods

The neuropathological methods used to diagnose the Alzheimer's disease, Parkinson's disease and DLB cases and to quantify the plaques, neurofibrillary tangles and Lewy bodies have been described previously (Perry *et al.*, 1990). Briefly, the fixed right cerebral hemisphere was sliced coronally and tissue was sampled from the midfrontal cortex, lateral parietal cortex, occipital cortex, temporal cortex, hippocampus, basal ganglia and brainstem. Paraffin sections 5 μm thick were cut and stained with haematoxylin and eosin for general histology, and used for the identification of Lewy bodies in the substantia nigra. In cortical areas, ubiquitin immunochemistry was used for the identification and quantification of Lewy bodies. Paraffin sections (20 μm) of the upper and lower midbrain were stained with cresyl fast violet and used for quantification of pigmented neurons in the substantia nigra. Paraffin sections (20 μm) of cortical areas were stained with a modification of Palmgren's silver technique for neurofibrillary tangle demonstration and quantification (Cross, 1982). The von Braunmühl silver impregnation technique was used to demonstrate plaques in 25-μm thick frozen sections cut from fixed tissue blocks adjacent to those taken for paraffin processing. For each of the four neocortical areas the mean tangle density was obtained by counting tangles in consecutive fields (0.61 mm^2 area) through the full width of the cortical ribbon in five randomly marked positions around the gyri (two at the crest, two in the mid-sulcal zone and one at the base of the sulcus); the mean plaque density was calculated from counts in fields (area = 3.1 mm^2), at five similarly marked points. In each case the tangle and plaque densities in the whole of the neocortex were both expressed as mean values per mm^2.

Brain sampling

For autoradiography, frozen tissue blocks were subdissected at –20°C, and 20 μm cryostat sections were cut and dried onto glass slides previously coated with Vectabond. All dopaminergic parameters were measured along the rostrocaudal axis, but because of tissue availability it was not possible to measure all parameters at all levels. Coronal levels in the anterior–posterior commissure axis (Fig. 1) were 0.5 cm apart and were designated according to Perry (Perry, 1993). Level 9 (not shown) marks the head of the caudate; level 10 the head of the putamen; level 11 the nucleus accumbens; level 12 the first appearance of the external globus pallidus and anterior commissure; level 13 the rostral limit of the temporal/frontal lobe junction; level 14 the rostral fornix, internal globus pallidus, amygdala and temporal horn of the lateral ventricle; and level 15 displays the lentiform nucleus formation, the mammillary body and the anterior nucleus of the thalamus.

Materials

[^{3}H]7-Hydroxy-dipropylaminotetralin (7-OH-DPAT) was supplied by Amersham (Amersham, UK), and NEN (London, UK) supplied [^{3}H]mazindol, [^{3}H]7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride (SCH23390) and [methoxy-^{3}H]raclopride. Butaclamol, desipramine, dopamine, *cis*-flupenthixol, ketanserin, nomifensine and *R*(–)-*N*-(3-propyl-1-propyl)-1-phenyl-2-aminopropane hydrochloride (PPAP) were supplied by Sigma (Poole, UK) and Vectabond by Vector Laboratories (Peterborough, UK). Other laboratory reagents were analytical grade, supplied by Sigma (Poole, UK).

Autoradiography protocols

For all ligands except [^{3}H]mazindol, cryostat sections were dried at room temperature for 2 h before storage at –70°C for up to 4 weeks prior to use. For mazindol binding, slides were dried for only 5 min prior to storage at –70°C overnight before use. At least four control cases were measured more than once at a particular coronal level to provide an additional control for consistency between each autoradiography experiment. Autoradiography protocols included preincubation in buffer to remove endogenous ligands and residual drugs, followed by incubation with radioactive ligand to determine total binding. Adjacent sections were incubated with the addition of a displacer to determine non-specific binding. Sections were given three washes in buffer, followed by a water dip to remove buffer salts, and dried under a stream of air prior to exposure to film. Triplicate determinations for both total and non-specific binding were made, and specific binding was calculated by subtracting the mean value of non-specific binding from the mean total binding.

Dopamine receptor autoradiography

Dopamine uptake sites

Dopamine uptake site binding was determined with 3 nM [^{3}H]mazindol, a concentration about half the dissociation

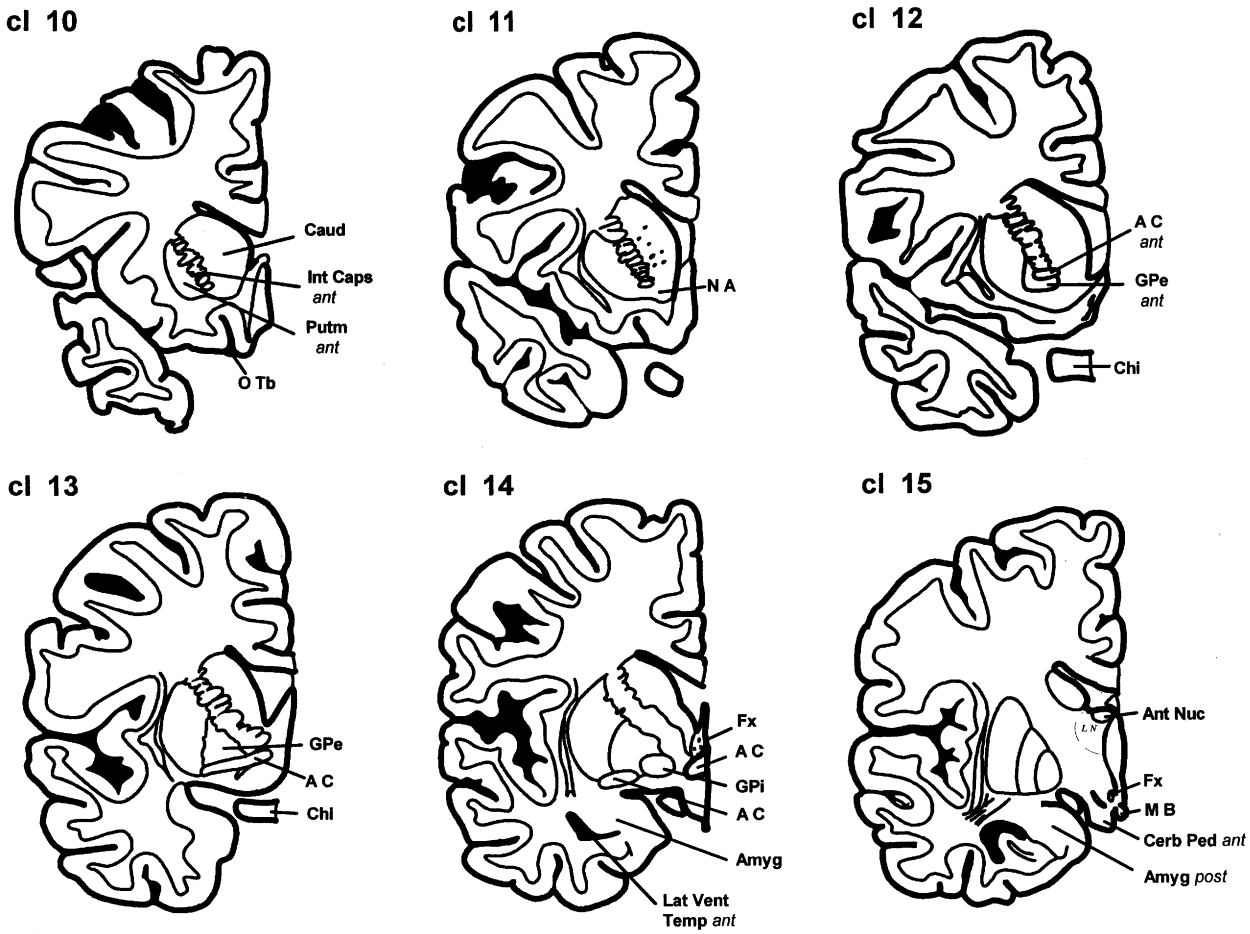

Fig. 1 Coronal levels of striatum, 0.5 cm apart; taken from the publication by R. Perry (Perry, 1993). AC = anterior commissure; Amyg = amygdala; Ant Nuc = anterior nucleus of thalamus; *ant* = anterior; Caud = caudate; Cerb Ped = cerebral peduncle; Chi = optic chiasma; cl = coronal level; Fx = fornix; GPe = external globus pallidus; GPi = internal globus pallidus; Int Caps = internal capsule; Lat Vent Temp = temporal horn of lateral ventricle; MB = mammillary body; NA = nucleus accumbens; O Tb = olfactory tubercle; *post* = posterior; Putm = putamen. Receptor densities were measured in the dorsal and ventral caudate and putamen, and in the nucleus accumbens.

constant K_d(h) reported previously (Singer *et al.*, 1991; Alexander *et al.*, 1992). Sections were removed from overnight storage at –70°C for 1 min prior to preincubation in 50 mM Tris–HCl (pH 7.9) at 4°C for 5 min, and were incubated with 3 nM [^{3}H]mazindol (specific activity = 24 Ci/mmol) in 50 mM Tris–HCl buffer (pH 7.9) containing 300 mM NaCl, 5 mM KCl and 100 nM desipramine (to block other monoamine uptake sites). Non-specific binding was defined in the presence of 100 μM nomifensine and was ~60% of total binding. Sections were given three 1-min washes at 4°C in buffer as used for the prewash.

Dopamine D1 receptors

For dopamine D1 receptor autoradiography, a modification of the method of Cortes (Cortes *et al.*, 1989) was used. Preincubation for 20 min at 4°C in 50 mM Tris–HCl (pH 7.4) was followed by incubation in 50 mM Tris–HCl (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$ and 1 nM [^{3}H]SCH23390 (specific activity = 71.1 Ci/mmol) with 100 nM ketanserin (to block 5-HT_2 binding sites) for 150 min at room temperature. A [^{3}H]SCH23390 concentration of 1 nM is slightly less than the K_d (dissociation constant) previously reported (Waddington and O'Boyle, 1987; Hall *et al.*, 1994). Non-specific binding was 30–40% of total binding, and was determined in the presence of 2 μM *cis*-flupenthixol. Slides were washed for a total of 9 min in 50 mM Tris–HCl (pH 7.4) buffer at 4°C.

Dopamine D2 receptors

For D2 receptors a protocol developed from the method of Kohler (Kohler and Radesater, 1986) was used. Sections were preincubated for 30 min in 50 mM Tris–HCl (pH 7.4) at room temperature and incubated with 3 nM [^{3}H]raclopride (specific activity = 69.5 Ci/mmol) in buffer composed of 50 mM Tris–HCl (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$ and 0.001% (w/v) ascorbic acid for 60 min at room temperature. This concentration of

[^{3}H]raclopride is above the K_d reported by Hall (Hall *et al.*, 1994) of 1.25 nM and within the range reported by Dean (Dean *et al.*, 1997) of 2–10 nM, in man. Non-specific binding was assessed in the presence of 1 μM butaclamol and was ~25% of total binding. Slides were washed for a total of 9 min in 50 mM Tris–HCl (pH 7.4) buffer at 4°C. The method for dopamine D2 receptors also measures a proportion of the smaller population of D3 receptors (Malmberg *et al.*, 1993), although Landwehrmeyer and colleagues (Landwehrmeyer *et al.*, 1993) found that [^{3}H]raclopride did not label D3 receptors in the islands of Calleja.

Dopamine D3 receptors

D3 autoradiography was carried out using modifications of the methods of Lévesque and colleagues (Lévesque *et al.*, 1992) and Herroelen and colleagues (Herroelen *et al.*, 1994), as previously described (Piggott *et al.*, 1999). Sections were preincubated twice for 10 min in 50 mM Tris–HCl (pH 7.4) containing 120 mM NaCl at room temperature to remove endogenous dopamine. The sections were incubated for 1 h at room temperature in 50 mM HEPES/NaOH buffer (pH 7.5) containing 1 mM EDTA, 100 nM PPAP (to block sigma sites) and 1 nM [^{3}H]7-OH-DPAT (specific activity = 152 Ci/mmol), a concentration similar to the K_d previously reported (Lévesque *et al.*, 1992; Herroelen *et al.*, 1994). Non-specific binding (10% of total binding) was in the presence of 10 μM dopamine. Sections were washed for a total of 9 min in 50 mM Tris–HCl containing 120 mM NaCl (pH 7.4) at 4°C.

Development and quantitative imaging of autoradiographs

The sections, together with autoradiographic standards (Amersham), were opposed to Hyperfilm ^{3}H (Amersham). Exposure times were 3 weeks for [^{3}H]mazindol, 1 month for [^{3}H]raclopride, 7 weeks for [^{3}H]SCH23390 and 3 months for [^{3}H]7-OH-DPAT. The resulting films were developed then scanned and quantified using a Lynx densitometry system (Applied Imaging, Sunderland, UK). Average values, calculated as fmol ligand bound per mg tissue equivalent, were measured by comparison with tritium standards (Amersham) at each coronal level in the areas of the dorsal and ventral caudate and putamen and in the nucleus accumbens.

Dopamine and homovanillic acid measurements

Dopamine and homovanillic acid (HVA) were measured in tissue homogenates of both caudate and putamen, dissected from the frozen tissue blocks, using high-performance liquid chromatography with electrochemical detection (Marshall *et al.*, 1994).

Statistical analysis

Statistical analysis was performed using Minitab for Windows, version 12. Comparison of binding between disease groups in the same striatal area at the same level was by one-way analysis of variance (ANOVA). Two-way ANOVA was used to compare binding in different areas at separate levels, and if no interaction between these factors was observed comparisons were then made by one-way ANOVA followed by Fisher's pairwise comparisons *post hoc* test, with the significance level set at $P \leqslant 0.05$, as suggested by Kinnear and Gray (Kinnear and Gray, 1994). The caudate and putamen were compared at similar levels and between pairs of levels by two-tailed *t* test. Assessment of correlation between neurochemical measures and demographic variables was calculated using Pearson's product moment correlation coefficient. Comparison of gender measurements was by two-tailed *t* test.

Results

The dopamine uptake site and D1, D2 and D3 receptor values (fmol/mg tissue; mean ± standard deviation) are shown in the dorsal and ventral caudate and putamen at the coronal levels measured in controls and in DLB, Parkinson's disease and Alzheimer's disease patients in Table 3. The graphs (Figs 2, 3, 4 and 6) illustrate the rostrocaudal distributions of the mean values of these parameters. Dopamine and HVA concentrations (pmol per mg protein; mean ± standard deviation) and the HVA : dopamine ratio are displayed in the rostrocaudal dimension (Fig. 7) in the caudate and putamen from controls and DLB, Parkinson's disease and Alzheimer's disease patients.

Dopamine uptake site (Fig. 2)

In control cases, binding to the dopamine uptake site was between 49 and 97 fmol/mg tissue in the putamen, and 62–122 fmol/mg in the caudate, with an increase in binding along the rostrocaudal axis in the caudate (especially ventrally), and higher binding in the ventral caudate than in the dorsal caudate and the putamen at coronal level 12 and coronal level 13, as previously described (Piggott *et al.*, 1999). Binding values were similar to those in previous reports (Singer *et al.*, 1991; Hurd and Herkenham, 1993) at equivalent ligand concentrations.

Comparison of disease groups (Fig. 2) in the dorsal putamen at coronal level 11 [$F(3,75) = 6.17$, $P = 0.001$] showed dopamine uptake site binding in Parkinson's disease cases to be reduced compared with Alzheimer's disease, DLB and controls, and binding in DLB cases to be slightly reduced compared with controls, with no significant differences between diseases in the coronal level 11 caudate. In the ventral putamen at coronal level 11, binding in Parkinson's disease cases was lower than in controls and Alzheimer's disease cases [$F(3,79) = 3.39$, $P = 0.011$].

Table 3 *Dopamine uptake site and D1, D2 and D3 receptor binding values (fmol per mg tissue, mean ± standard deviation) in dorsal and ventral caudate and putamen at each coronal level in controls, DLB, Parkinson's disease and Alzheimer's disease*

Dopamine uptake site		Coronal level	Dorsal caudate	Ventral caudate	Dorsal putamen	Ventral putamen	Nucleus accumbens
	Controls	10	62.1 ± 13.1	64.1± 29.7	48.7 ± 16.4	73.2 ± 38.7	
		11	88.5 ± 27.1	89.3 ± 21.6	79.9 ± 21.5	78.1 ± 22.9	67.1 ± 17.1
		12	90.2 ± 27.6	109.1 ± 18.7	90.0 ± 17.3	91.7 ± 13.1	
		13	100.1 ± 25.1	121.7 ± 24.4	94.2 ± 26.3	97.0 ± 26.5	
		14	101.8 ± 41.6	112.8 ± 51.6	82.7 ± 28.9	94.4 ± 28.2	
	DLB	11	81.1 ± 41.4	96.8 ± 43.2	59.3 ± 26.5	62.8 ± 31.8	65.1 ± 36.7
		12	51.6 ± 21.2‡‡	87.4 ± 33.1	38.3 ± 16.3‡‡‡	53.6 ± 32.5	
		13	57.4 ± 38.6†	83.2 ± 42.7†	38.4 ± 28.8†	45.1 ± 26.9†	
	PD	10	39.1 ± 28.9‡‡	53.2 ± 35.9	30.1 ± 25.6	39.3 ± 27.8	
		11	54.7 ± 46.4	78.2 ± 53.5	35.5 ± 28.4§	42.9 ± 25.1†	56.8 ± 39.8
		12	28.1 ± 22.1‡‡	56.5 ± 21.0‡‡	18.8 ± 25.3‡‡	27.2 ± 22.2‡‡	
		13	37.2 ± 18.3‡	86.4 ± 27.1‡	22.6 ± 17.3‡	25.1 ± 21.2‡	
	AD	9	76.0 ± 29.4	83.8 ± 34.2			
		11	76.2 ± 37.5	82.2 ± 37.4	68.5 ± 34.5	76.4 ± 38.6	64.0 ± 32.4
		13	105.1 ± 36.6	122.2 ± 42.3	95.9 ± 39.8	108.5 ± 38.5	
D1	Controls	10	33.4 ± 7.1	32.6 ± 9.1	37.4 ± 7.1	32.8 ± 9.2	
		11	29.9 ± 5.7	29.2 ± 8.2	36.0 ± 7.2	29.4 ± 7.1	34.7 ± 9.7
		13	36.4 ± 10.7	33.8 ± 9.1	31.2 ± 9.0	25.8 ± 6.8	
		14	37.4 ± 2.5	35.0 ± 2.4	28.6 ± 9.1	26.9 ± 12.0	
	DLB	10	29.2 ± 11.7	33.2 ± 9.8	34.2 ± 10.5	36.5 ± 12.5	
		11	30.9 ± 8.7	30.1 ± 7.8	32.6 ± 7.2	31.7 ± 7.3	36.8 ± 8.2
		13	29.0 ± 9.0	28.4 ± 10.6	31.6 ± 11.1	27.7 ± 10.6	
	PD	10	32.6 ± 9.8	33.4 ± 10.6	32.3 ± 7.1	30.8 ± 17.4	
		11	30.3 ± 7.1	29.9 ± 9.0	36.4 ± 6.9	32.1 ± 9.3	32.0 ± 10.8
		12	34.1 ± 17.6	32.2 ± 17.4	33.7 ± 18.2	30.6 ± 14.5	
		13	45.8 ± 9.2#	42.1 ± 1.5#	40.5 ± 11.7#	38.0 ± 7.7#	
	AD	10	35.2 ± 8.0	37.3 ± 12.0	25.9 ± 16.4	20.7 ± 21.0	
		11	31.6 ± 14.4	26.0 ± 14.6	36.0 ± 14.5	28.0 ± 13.3	32.0 ± 20.2
		13	29.0 ± 12.4	27.2 ± 14.5	27.4 ± 10.2	22.8 ± 12.0	
D2	Controls	10	19.4 ± 7.3	19.8 ± 8.3	22.3 ± 9.9	21.2 ± 9.9	
		11	19.3 ± 10.4	19.1 ± 12.1	23.1 ± 12.0	22.0 ± 11.8	24.8 ± 14.6
		13	29.2 ± 9.1	24.5 ± 8.2	27.6 ± 10.4	25.7 ± 9.8	
		15	31.8 ± 14.1	32.1 ± 13.7	27.4 ± 11.7	28.0 ± 11.1	
	DLB	11	18.1 ± 4.9‡	17.0 ± 5.6	20.5 ± 6.3	20.6 ± 7.0	21.5 ± 7.9
		13	16.9 ± 11.7§	18.0 ± 8.9	19.5 ± 12.9	24.0 ± 16.2	
		14	21.1 ± 13.0	18.4 ± 13.6	23.6 ± 14.0	22.8 ± 14.0	
	PD	10	34.0 ± 4.6	30.7 ± 7.9	39.1 ± 4.3	36.9 ± 8.5	
		11	34.7 ± 7.5#	28.5 ± 5.4#	41.4 ± 13.3#	36.1 ± 10.1#	37.2 ± 13.2#
		12	33.7	35.1	38.5	40.2	
		13	32.8 ± 10.4	28.2 ± 6.1	35.3 ± 11.1**	35.6 ± 9.5**	
	AD	11	24.4 ± 11.4	21.3 ± 10.8	24.9 ± 12.5	23.9 ± 11.7	20.3 ± 13.1
		14	31.4 ± 13.4	28.0 ± 12.7	29.1 ± 13.2	31.3 ± 15.7	
D3	Controls	10	5.8	9.8			
		11	4.5 ± 1.0	6.0 ± 1.6	6.9 ± 1.2	10.9 ± 1.7	16.3 ± 2.7
		12	4.3 ± 1.2	5.2 ± 0.9	6.7 ± 1.2	11.3 ± 0.9	
		14	3.4 ± 0.5	3.9 ± 0.5	4.1 ± 1.04	5.0 ± 1.4	
	DLB	10	4.4 ± 1.7	9.6 ± 2.6	6.2 ± 1.9	7.3 ± 2.0	
		11	4.9 ± 2.3	7.7 ± 3.1	8.2 ± 3.4	12.0 ± 3.0	16.4 ± 3.8
		13	4.6 ± 1.9	5.6 ± 2.0	8.2 ± 2.6	11.8 ± 2.7	
		14	3.5 ± 1.1	4.4 ± 1.2	5.1 ± 3.6	5.5 ± 3.9	
	PD	10	4.8 ± 2.0	9.7 ± 3.7	6.7 ± 0.8	10.3 ± 2.1	
		11	4.2 ± 0.7	6.3 ± 0.7	6.3 ± 0.4	11.0 ± 0.8	13.0 ± 2.3
		13	3.4 ± 1.5	4.6 ± 1.6	4.4 ± 2.4	6.7 ± 3.4¶	
	AD	10	5.5 ± 3.1	8.9 ± 5.9	9.2 ± 0.6	12.3 ± 2.9	
		11	6.5 ± 2.0††	8.9 ± 2.8	9.3 ± 3.5††	13.5 ± 5.3††	15.6 ± 5.4
		13	5.2 ± 1.8	5.0 ± 1.1	6.8 ± 2.2	8.4 ± 1.8	

Significant differences between disease groups by ANOVA followed by Fisher's *post hoc* comparison of means with significance set at $P < 0.05$ are indicated. AD = Alzheimer's disease; PD = Parkinson's disease. *Significantly lower than controls; †significantly lower than control and Alzheimer's disease cases; ‡significantly lower than Alzheimer's disease cases; §significantly lower than control, DLB and Alzheimer's disease cases; ¶significantly lower than control and DLB cases; #significantly higher than control, DLB and Alzheimer's disease cases; **significantly higher than control and DLB cases; ††significantly higher than control and Alzheimer's disease cases; ‡‡, ‡‡‡significantly lower than controls at $P < 0.05$ and $P < 0.01$, respectively (*t* test).

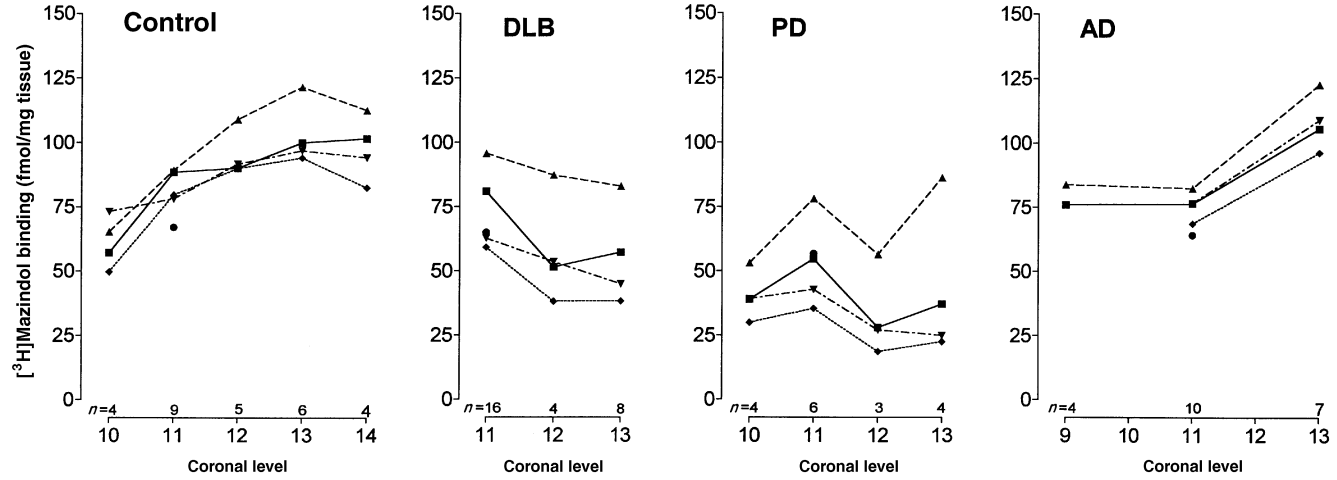

Fig. 2 [^{3}H]Mazindol binding to dopamine uptake sites (mean of *n* as indicated, fmol/mg tissue at each point) in the dorsal and ventral caudate and putamen in controls, DLB patients, Parkinson's disease (PD) patients and Alzheimer's disease (AD) patients. In Parkinson's disease, coronal level 13 represents the mean value of three cases at coronal level 13 and one case at coronal level 14. ■, dorsal caudate; ▲, ventral caudate; ◆, dorsal putamen; ▼, ventral putamen; ●, nucleus accumbens.

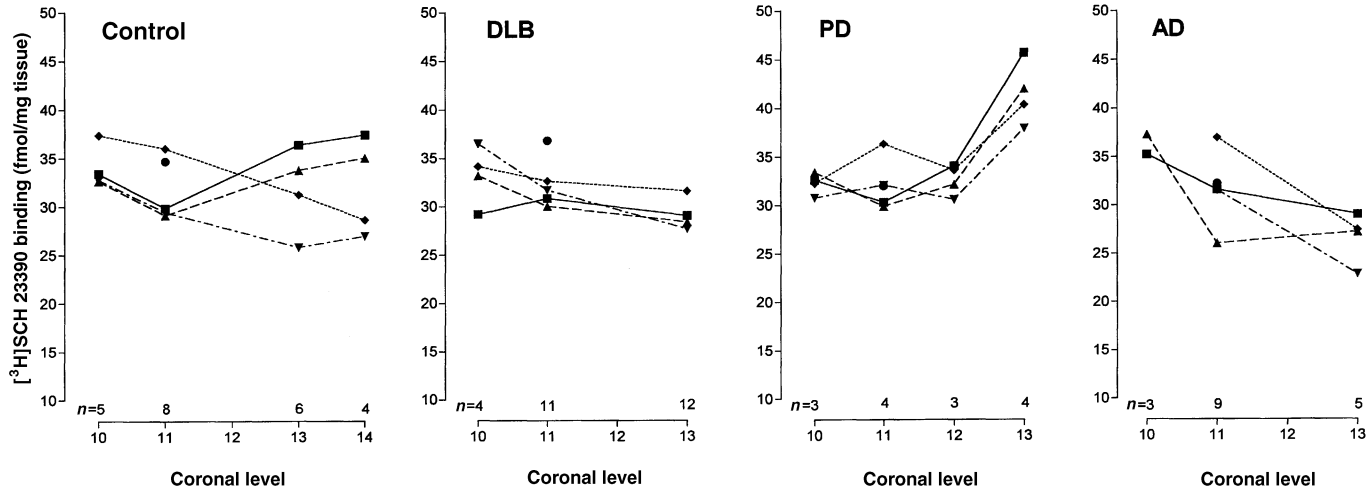

Fig. 3 [^{3}H]SCH23390 binding to dopamine D1 receptors (mean of *n* as indicated, fmol/mg tissue) in the dorsal and ventral caudate and putamen in controls, DLB patients, Parkinson's disease (PD) patients and Alzheimer's disease (AD) patients.

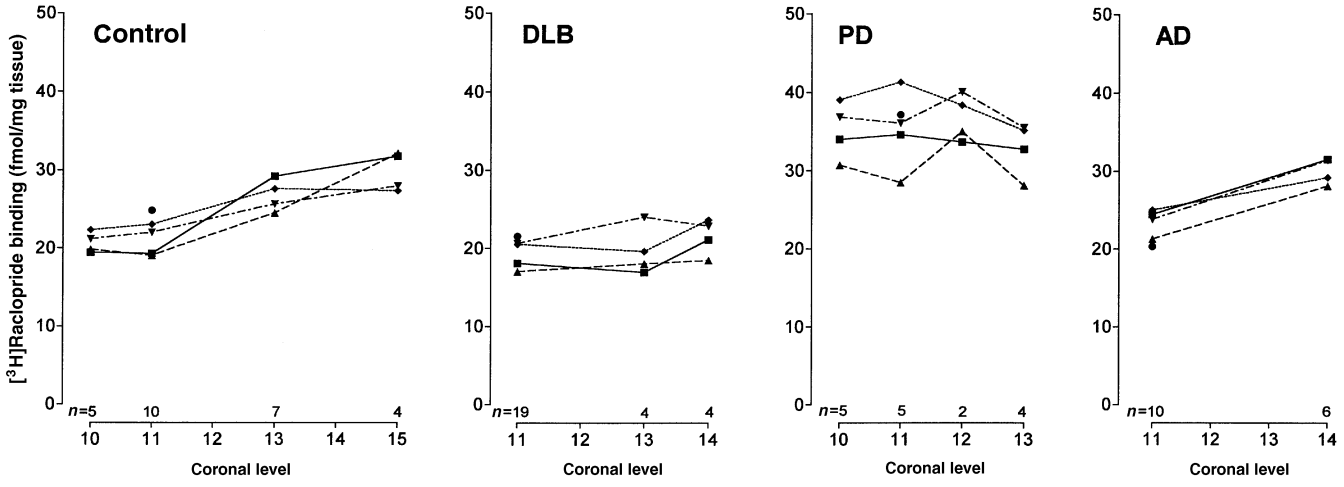

Fig. 4 [^{3}H]Raclopride binding to dopamine D2 receptors (mean of *n* as indicated, fmol/mg tissue) in the dorsal and ventral caudate and putamen in controls, DLB patients, Parkinson's disease (PD) patients and Alzheimer's disease (AD) patients.

There were no significant differences between disease groups in the nucleus accumbens. At coronal level 13, the Parkinson's disease and DLB groups had similarly low levels of dopamine uptake sites compared with control and Alzheimer's disease cases in the dorsal caudate [$F(3,45) = 10.98$, $P < 0.001$] and ventral caudate [$F(3,42) = 4.13$, $P = 0.012$), dorsal putamen [$F(3,48) = 18.36$, $P < 0.001$] and in the ventral putamen [$F(3,46) = 20.99$, $P < 0.001$].

In DLB at coronal level 12, dopamine uptake site binding was significantly reduced compared with controls in the dorsal caudate (t test, $P = 0.026$) and in the dorsal putamen ($P = 0.001$). There was no increasing rostrocaudal gradient of dopamine uptake site binding in DLB cases as in the controls; in the dorsal caudate there was a trend to a decreasing rostrocaudal gradient, which approached significance (correlation coefficient $r = –0.35$, $0.1 > P > 0.05$).

In Parkinson's disease, dopamine uptake site binding was reduced at all levels, being significantly lower than in controls in the dorsal caudate at coronal level 10 (t test, $P = 0.03$) and in the caudate and putamen at coronal level 12 ($P = 0.019$ and 0.02, respectively). The greatest losses of dopamine uptake sites in Parkinson's disease were at caudal coronal levels (75% reduction in the putamen at coronal level 13) and the least reduction was in the ventral caudate; there was no significant gradient of binding in the rostrocaudal dimension in any area.

In Alzheimer's disease there was no significant deviation from control dopamine uptake site binding at any level, with an increasing rostrocaudal gradient which was almost significant in the ventral caudate ($r = 0.375$, $P \approx 0.05$).

There were no significant age or post-mortem delay differences between disease groups [$F(3,72) = 1.5$, $P = 0.22$ and $F(3,72) = 0.84$, $P = 0.48$, respectively]. Significant changes with demographic variables within groups were a decline in dopamine uptake site binding in DLB cases with age, which was most significant in the ventral caudate at posterior levels ($r = –0.56$, $n = 11$, $P < 0.05$). In Parkinson's disease there was a tendency to a reduced concentration of dopamine uptake sites with disease duration, which reached significance in the ventral caudate at posterior levels ($r = –0.77$, $n = 7$, $P < 0.02$). In the Alzheimer's disease group there was a significant negative correlation with increasing age in all Alzheimer's disease cases, especially in the caudate nucleus and also when the analysis was restricted to coronal level 11 (dorsal caudate, $r = –0.758$, $n = 10$, $P < 0.001$; ventral caudate, $r = –0.697$, $n = 10$, $P < 0.01$). There was also a significant decline in dopamine uptake sites with increasing age of onset of Alzheimer's disease, particularly in the caudate ($r = –0.53$, $n = 14$, $P < 0.05$, coronal level 11 only).

D1 receptor (Fig. 3)

In control cases, D1 receptor binding was ~30–35 fmol/mg tissue, comparable to other published values (Cortes *et al.*, 1989; De Keyser *et al.*, 1990); it was higher in the caudate compared with the putamen at posterior levels and displayed a rostrocaudal decline in the putamen which was significant dorsally, as previously reported (Piggott *et al.*, 1999). The only difference in D1 binding in disease groups was between Parkinson's disease and all other groups at coronal level 13. A two-way ANOVA at coronal level 13 revealed that there was no significant difference between areas but a significant difference between groups [$F(3,96) = 9.69$, $P < 0.001$], with no group × area interaction. Subsequent one-way ANOVA showed a significant group difference [$F(3,227) = 14.92$, $P < 0.001$] and *post hoc* analysis (with significance level set at $P < 0.05$) showed the Parkinson's disease group to have higher D1 binding than all other groups at coronal level 13. This was particularly so in the ventral putamen (t test, $P = 0.014$ compared with controls).

With respect to demographic variables, in the control group there was a significant negative correlation of D1 binding with age in the putamen at coronal level 11 ($r = –0.83$, $n = 8$, $P < 0.01$). In DLB cases there was a significant negative correlation with age at coronal levels 10 and 11 in the ventral caudate and putamen ($r = –0.5$, $n = 15$, $P < 0.05$) and in the dorsomedial caudate ($r = –0.62$, $n = 15$, $P < 0.01$). There was a significant decline in D1 binding with age of onset of DLB in the caudate ($r = –0.53$, $n = 14$, $P < 0.05$). D1 binding declined with disease duration in Parkinson's disease; the decline was significant in the posterior caudate ($r = –0.75$, $n = 6$, $P < 0.05$).

D2 receptor (Fig. 4)

D2 binding in controls of ~20–30 fmol/mg tissue was in a similar range to other published values, allowing for different ligand concentrations (Hall *et al.*, 1994; Dean *et al.*, 1997). D2 receptor binding in controls was relatively uniform in the dorsoventral and lateromedial dimensions, with more variation in the increasing gradient rostrocaudally (Fig. 4). At coronal levels 13 and 15, D2 binding was higher than at coronal levels 10 and 11, with no difference between the caudate and putamen except at coronal level 11, where D2 binding in the putamen was higher, as previously reported (Piggott *et al.*, 1999).

D2 binding was significantly different between disease groups [$F(3,744) = 49.58$, $P < 0.001$] and between areas [$F(9,744) = 3.05$, $P = 0.001$], with no significant interaction term. At coronal level 11, D2 binding was elevated in Parkinson's disease above control, DLB and Alzheimer's disease values in the dorsal caudate [$F(3,80) = 10.93$, $P < 0.001$], ventral caudate [$F(3,88) = 4.99$, $P = 0.003$), dorsal putamen [$F(3,84) = 11.01$, $P < 0.001$], ventral putamen [$F(3,90) = 6.97$, $P < 0.001$] and nucleus accumbens [$F(3,72) = 4.98$, $P = 0.003$); in the dorsal caudate at coronal level 11, D2 binding was significantly lower in DLB than in Alzheimer's disease [$F(3,80) = 10.93$, $P < 0.001$]. At caudal levels (coronal levels 12 and 13), D2 binding in Parkinson's disease was elevated above control and DLB values in the

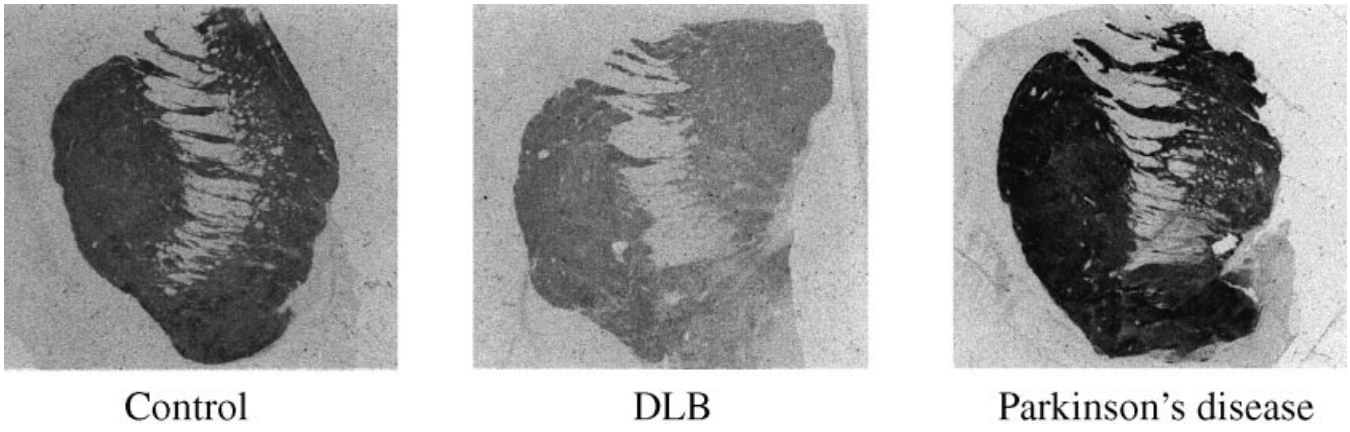

Fig. 5 Autoradiographs of [^{3}H]raclopride binding to D2 receptors in the striatum in a control case, a DLB case and a Parkinson's disease case at coronal level 11, showing higher binding in Parkinson's disease and lower binding in DLB patients compared with the control group.

dorsal putamen [$F(3,68) = 3.62$, $P = 0.017$] and the ventral putamen [$F(3,66) = 3.15$, $P = 0.03$]. In addition, in DLB patients the level of D2 binding in the dorsal caudate at coronal levels 13 and 14 was lower than in controls and in Alzheimer's disease and Parkinson's disease patients [$F(3,62) = 4.19$, $P = 0.009$]. Figure 5 shows that D2 binding at coronal level 11 was lower in DLB and higher in Parkinson's disease than in controls. There was no difference in D2 binding between controls and Alzheimer's disease at rostral or caudal levels, and in Alzheimer's disease a rostrocaudal and dorsoventral distribution similar to that of controls was found. There was much less rostrocaudal variation in DLB and Parkinson's disease.

Significant changes with demographic variables included an effect of gender in DLB, with lower D2 binding in males in the ventral caudate and ventral putamen (*t* test, $P = 0.01$ and 0.022, respectively). There was a trend for decreased D2 binding with longer duration of DLB in all areas ($r = –0.38$, $n = 23$, $0.1 > P > 0.05$ in the dorsal caudate). A reduction in D2 binding with age in Parkinson's disease was most significant in the rostral ventral putamen ($r = –0.58$, $n = 10$, $P < 0.05$). There was a trend in all striatal areas to a lower level of D2 binding with older age of onset in Parkinson's disease, which reached significance in the ventral putamen ($r = –0.56$, $n = 10$, $P < 0.05$). Disease duration in Alzheimer's disease was associated with a significant increase in D2 receptors in the rostral putamen ($r = 0.64$, $n = 10$, $P < 0.05$).

D3 receptors (Fig. 6)

D3 binding was 5–20 fmol/mg tissue, similar to or higher than that reported in other studies, none of which used a method identical to that presently described (Herroelen *et al.*, 1994; Gurevich *et al.*, 1997). D3 binding was distributed unevenly, with the highest density binding ventrally, as previously described (Piggott *et al.*, 1999). Analysis of all groups at coronal level 11 showed significantly different D3 binding between disease groups [$F(3,95) = 4.78$, $P = 0.004$] and in striatal areas [$F(4,95) = 53.38$, $P < 0.001$], but with no significant interaction. There was no significant difference in D3 binding in DLB compared with controls in any area. D3 binding in the dorsal caudate and putamen in Alzheimer's disease at coronal level 11 was elevated compared with controls and Parkinson's disease cases [$F(3,65) = 4.42$, $P = 0.007$]. Comparing the nucleus accumbens only, there was no significant difference between groups. Comparison of groups at coronal level 13 showed a significant group [$F(3,58) = 6.41$, $P = 0.001$] and area difference [$F(3,58) = 21.0$, $P < 0.001$] and no significant interaction term. At coronal level 13, D3 binding in the caudate and putamen combined showed significant disease differences [$F(3,70) = 2.81$, $P = 0.046$], *post hoc* analysis ($P < 0.05$) showing significantly reduced D3 binding in Parkinson's disease compared with controls and DLB cases, particularly in the ventral putamen [$F(3,13) = 4.04$, $P = 0.03$].

Significant changes with demographic variables were that in DLB with increasing disease duration in the posterior caudate, D3 binding declined (dorsal caudate, $r = –0.72$, $n = 7$, $P < 0.05$; ventral caudate, $r = –0.78$, $n = 7$, $P < 0.02$). In Parkinson's disease there was a tendency for D3 binding to decline with age in the anterior dorsal putamen ($r = –0.7$, $n = 8$, $0.1 > P > 0.05$).

Dopamine concentration and metabolism (Fig. 7)

The dopamine concentration in control cases was greatest at coronal levels 11–13 in the caudate and putamen while the concentration of HVA displayed no gradient in the caudate but peaked in the putamen at coronal level 12. The HVA : dopamine ratio in the posterior striatum was higher in the putamen than in the caudate, as previously reported (Piggott *et al.*, 1999).

Comparing disease groups, dopamine was significantly reduced in the caudate in DLB and Parkinson's disease relative to controls and Alzheimer's disease cases [$F(3,91) =$

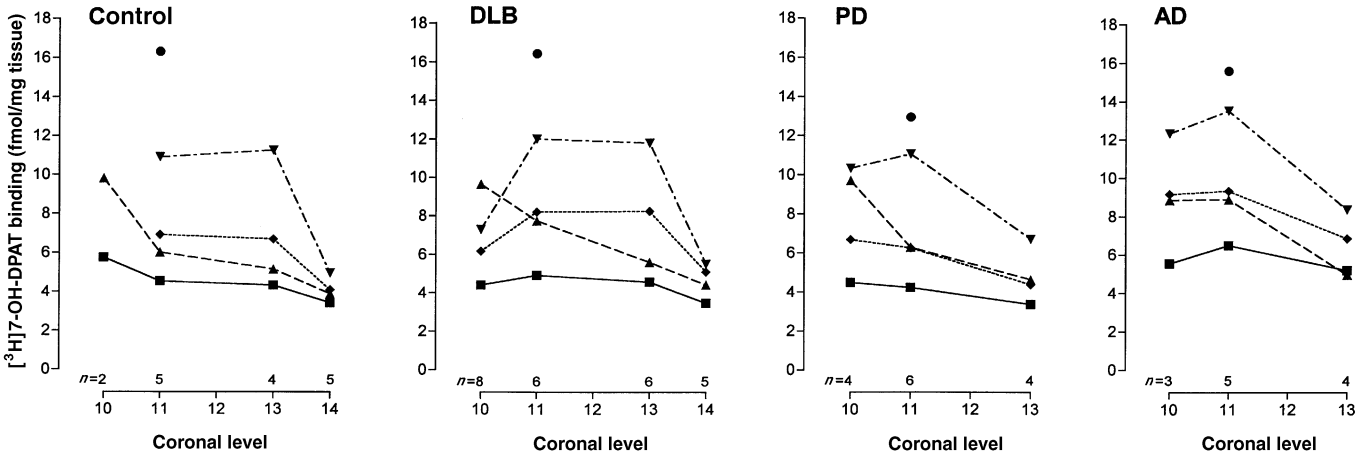

Fig. 6 [^{3}H]7-OH-DPAT binding to dopamine D3 receptors (mean of *n* as indicated, fmol/mg tissue) in the dorsal and ventral caudate and putamen in controls, DLB patients, Parkinson's disease (PD) patients and Alzheimer's disease (AD) patients.

12.02, $P < 0.001$]. The loss of dopamine in the caudate in DLB was uniform along the rostrocaudal axis (42–46%), but in Parkinson's disease the dopamine reduction in the posterior caudate was much more extensive (86%, compared with 60% rostrally). In the putamen, the dopamine concentration was significantly reduced in both DLB and Parkinson's disease compared with controls and Alzheimer's disease cases [$F(3,80) = 18.38$, $P < 0.001$]. In the putamen in both DLB and Parkinson's disease, dopamine loss was more extensive caudally. In the putamen in DLB, dopamine was reduced by 35% rostrally and 72% caudally, while in the putamen in Parkinson's disease dopamine was reduced by 79% rostrally and 90% caudally.

In DLB, HVA was extensively reduced, to an extent similar to that in Parkinson's disease, in both the caudate and the putamen [group comparisons: $F(3,90) = 5.89$, $P = 0.001$ and $F(3,80) = 5.31$, $P = 0.002$, respectively]. HVA concentrations in Parkinson's disease and DLB were significantly lower than in controls and Alzheimer's disease cases in the putamen, and significantly lower than in controls in the caudate. The HVA : dopamine ratio was not significantly different in DLB compared with controls, although it tended to be raised in the caudal putamen, while the HVA : dopamine ratio in Parkinson's disease was highly elevated in all areas, especially in the putamen, where it was 6–13 times higher than in controls [$F(3,80) = 8.82$, $P < 0.001$].

In Alzheimer's disease the dopamine content in the putamen was significantly higher than in controls [$F(3,80) = 18.38$, $P < 0.001$] both rostrally and caudally, while the HVA concentration was slightly less than in controls in the caudate [$F(3,90) = 5.89$, $P = 0.001$]. The HVA : dopamine ratios in Alzheimer's disease were not significantly different from those in controls in the caudate and putamen, but the standard deviation of the ratio was larger in the caudal putamen.

Significant demographic influences were that in DLB there was a loss of dopamine with age in the caudate ($r = –0.46$, $n = 25$, $P < 0.02$), longer disease duration tended to result in an increased HVA : dopamine ratio in the putamen in DLB, and increased age of onset of DLB was positively correlated with HVA concentration in the caudate ($r = 0.51$, $n = 15$, $P < 0.05$) and putamen ($r = 0.8$, $n = 15$, $P < 0.001$) at coronal levels 11–13. Age in Parkinson's disease was inversely correlated with dopamine and HVA concentrations in the caudate ($r = –0.51$, $n = 18$, $P < 0.01$ and $r = –0.7$, $n = 12$, $P < 0.01$, respectively, at coronal levels 11–13). There was also a significant decline in HVA concentration with increasing Parkinson's disease duration in the caudate and putamen ($r = –0.69$, $n = 12$, $P < 0.01$ and $r = –0.73$, $n = 12$, $P < 0.01$, respectively, at coronal levels 11–13). In Alzheimer's disease there was a significant decline in dopamine concentration with increasing age in the caudate and putamen ($r = –0.73$, $n = 15$, $P < 0.01$ and $r = –0.52$, $n = 13$, $P < 0.05$, respectively, at coronal levels 11–13). In Alzheimer's disease, dopamine and HVA concentrations declined with increasing disease duration in the putamen ($r = –0.78$, $n = 8$, $P < 0.01$ and $r = –0.62$, $n = 8$, $0.1 > P > 0.05$, respectively, at coronal level 11).

Discussion

The principal findings of the present study of clinically and pathologically assessed cases of DLB, Parkinson's disease and Alzheimer's disease were of a differential loss of dopamine uptake sites in DLB, which was restricted to levels of the striatum caudal to the anterior commissure, with reduction at rostral levels limited to the dorsal putamen. D2 receptor expression was also distinctive in DLB, being much lower than in Parkinson's disease and reduced caudally compared with controls and Alzheimer's disease. These differences in the neurochemical pathology of DLB may explain some clinical features of the disease, including the spontaneous extrapyramidal symptoms which respond poorly to L-dopa medication, and marked neuroleptic sensitivity. The distinct neurochemical profile may be of diagnostic value using *in vivo* imaging.

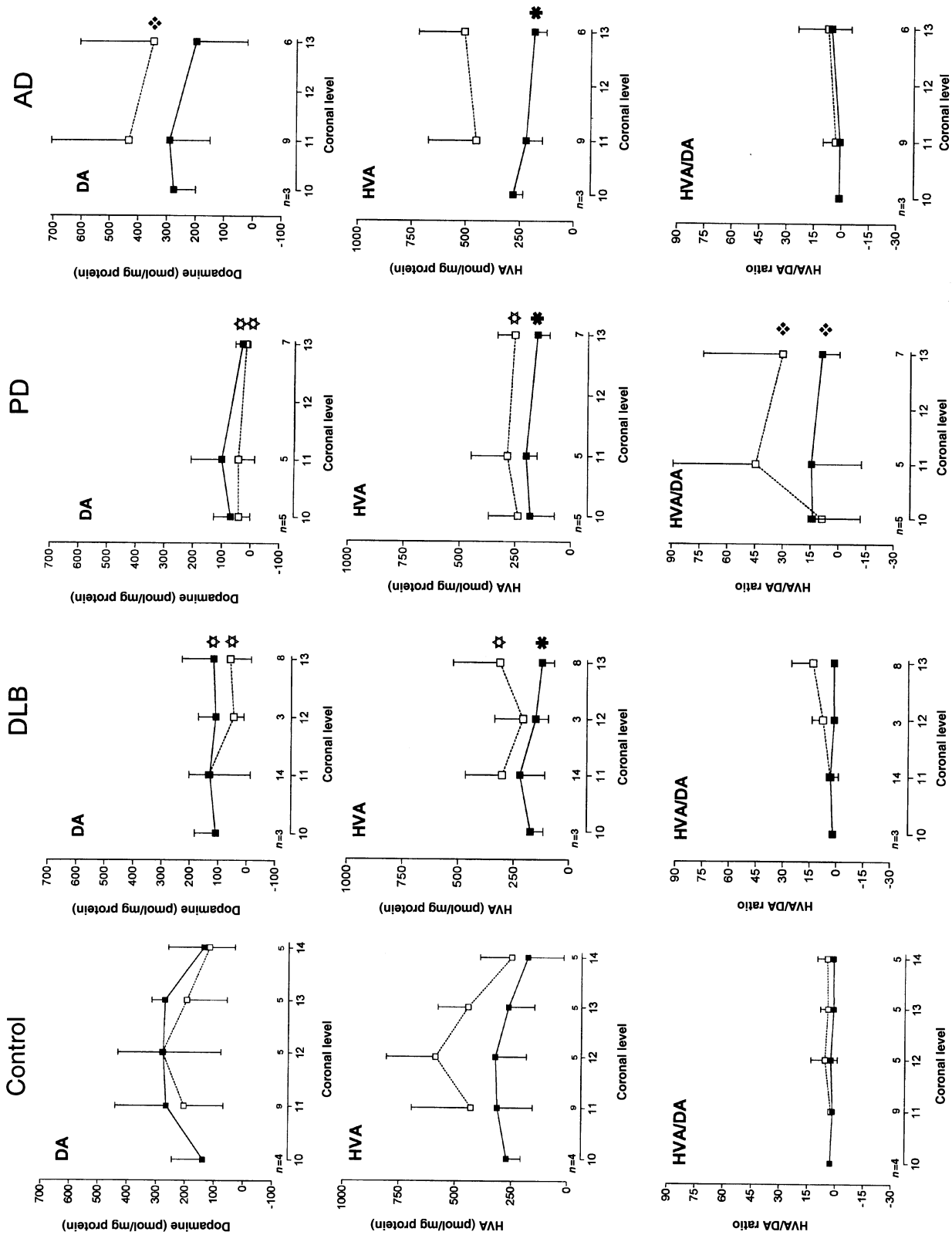

Fig. 7 Rostrocaudal distribution of dopamine and HVA (mean ± standard deviation of n as indicated, pmol/mg protein), and HVA : dopamine (DA) ratio in controls, DLB patients, Parkinson's disease (PD) patients and Alzheimer's disease (AD) patients. Significant differences between disease groups by ANOVA and Fisher's *post hoc* comparison of means with significance set at $P < 0.05$ are indicated by ✡, significantly lower than controls and Alzheimer's disease cases; ✱, significantly lower than controls; ❖, significantly higher than controls, DLB and Alzheimer's disease cases. ■, Caudate; □, putamen.

Dopamine uptake sites

In DLB, reduced dopamine uptake sites were restricted to levels posterior to the anterior commissure, affecting the putamen and dorsal caudate rather than the ventral caudate. This may be similar to the pattern of loss in very early Parkinson's disease (Wilson *et al.*, 1996; Booij *et al.*, 1997). In contrast, dopamine uptake sites were found to be reduced at all coronal levels of the putamen in Parkinson's disease and in all of the caudate posterior to the anterior commissure, consistent with many previous reports both *in vitro* (Guttman, 1987; Wilson *et al.*, 1996) and *in vivo* (Antonini *et al.*, 1995; Brucke *et al.*, 1997; Tatsch *et al.*, 1997; Tissingh *et al.*, 1998). In early drug-naïve Parkinson's disease, dopamine uptake site losses measured by single photon emission computerized tomography (SPECT) imaging are bilateral even in unilateral disease (Tissingh *et al.*, 1998). Reductions in markers of dopaminergic terminals in Parkinson's disease are well reported, with greater loss in the caudal putamen initially and later reductions spreading rostrally and involving the caudate (Kish *et al.*, 1988; Murray *et al.*, 1995; Wilson *et al.*, 1996), similar to the pattern seen in the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) monkey (Moratalla *et al.*, 1992). The threshold of reduction in dopamine uptake sites for the appearance of mild parkinsonism may be quite low—below 20% in the putamen overall and ~50% in the posterior putamen, as measured *in vivo* (Eising *et al.*, 1997; Guttman *et al.*, 1997)—suggesting that the loss of dopamine uptake sites in DLB is sufficient to evoke extrapyramidal symptoms.

In Alzheimer's disease, mazindol binding to the dopamine uptake site was not reduced at all, in agreement with previous reports showing little reduction *in vitro* (Murray *et al.*, 1995) or *in vivo* (Tyrrell *et al.*, 1990; Donnemiller *et al.*, 1997), but in contrast to reports of reduced dopamine uptake sites (Rinne *et al.*, 1998; Sahlberg *et al.*, 1998) that were correlated with the severity of extrapyramidal symptoms. In the present study, older age of onset of Alzheimer's disease was associated with lower dopamine uptake site density, perhaps reflecting a decline in binding with age or more extrapyramidal symptoms with older age of Alzheimer's disease onset. The pathological correlates of extrapyramidal features in Alzheimer's disease may also be in other parts of the basal ganglia–thalamocortical circuit or, more controversially, in neurofibrillary tangles in the substantia nigra (Liu *et al.*, 1997).

The decline in dopamine uptake sites with disease duration in Parkinson's disease and Alzheimer's disease was not seen significantly in DLB, which may parallel the observation that extrapyramidal symptoms progress only slowly during the course of the disease (Ballard *et al.*, 1998*b*). Imaging of dopamine uptake has been shown to correlate with disease duration and increasing movement disability (Tatsch *et al.*, 1997). Dopamine uptake sites tended to be reduced with age, especially in the caudate in DLB and Alzheimer's disease, consistent with previous reports over more extended age ranges (De Keyser *et al.*, 1990; Rinne *et al.*, 1998; Volkow *et al.*, 1998). A proportion of the cases received L-dopa therapy or neuroleptic treatment, and the possible effects of these must be considered. Longer duration of L-dopa therapy may increase the rate of loss of dopamine uptake sites as substantia nigra neurons are lost, due to the metabolic cost of processing L-dopa (Kopin, 1993; Watts, 1997), which may contribute to the reduction in uptake site binding in Parkinson's disease with increased disease duration. Conversely, however, L-dopa treatment of MPTP monkeys has been reported to increase mazindol binding (Rioux *et al.*, 1997). Neuroleptic treatment is reported not to affect dopamine uptake sites in long-term therapy in schizophrenia (Czudek and Reynolds, 1989) and in rats, but may tend to reduce presynaptic markers in older groups of DLB and Alzheimer's disease patients, particularly in the caudate (Perry *et al.*, 1998).

D2 receptor binding

D2 receptor binding was elevated in Parkinson's disease, especially rostrally, while in DLB values were lower than in Parkinson's disease at all coronal levels, and lower than in controls caudally. In Alzheimer's disease, D2 receptor densities in the striatum were unchanged. Consequently, while D2 binding was elevated in Parkinson's disease at all levels, the elevation was relatively greater rostrally; conversely, in DLB the deficit in D2 binding was relatively greater caudally, compared with controls. Several reports have shown raised D2 binding in early Parkinson's disease, as measured by SPET or PET (Giobbe *et al.*, 1993; Antonini *et al.*, 1994, 1997*a*; Reiche *et al.*, 1995), and it has been suggested that *in vivo* imaging may help to differentiate idiopathic Parkinson's disease from other parkinsonism-plus syndromes like multiple system atrophy and progressive supranuclear palsy (Tissingh *et al.*, 1997), in which, as in DLB, reduced D2 density occurs, and L-dopa therapy is less effective. In addition, it has been reported that patients with multiple system atrophy are at risk of developing a neuroleptic malignant syndrome-like condition (Konagaya *et al.*, 1997). How early in the Parkinson's disease process D2 elevation occurs or whether a threshold of dopamine loss needs to be reached is not clear. Antonini and colleagues (Antonini *et al.*, 1995) found in a PET study that D2 elevation correlated with reduced [^{18}F]fluoro-L-dopa uptake in early Parkinson's disease, and they did not have any patients so early in disease that they did not show D2 upregulation. Rinne and colleagues (Rinne *et al.*, 1993) found D2 upregulation to be present at the time of diagnosis. Striatal dopamine concentrations are required to be significantly reduced before clinical symptoms become apparent, and the tendency for D2 receptors to upregulate as a compensatory measure will mask clinical symptoms in the earliest phase. L-Dopa therapy and longer Parkinson's disease duration (over years rather than months) have been found to lower previously elevated D2 receptors *in vivo* (Antonini *et al.*, 1997*b*), although levels have been

reported to remain high for >36 months with dopaminergic therapy (Hierholzer *et al.*, 1998). In DLB, D2 binding was reduced by 17% in the caudal putamen compared with controls, in an area where dopamine uptake sites were reduced to 52% of the control value, and therefore dopamine terminals are probably depleted sufficiently for D2 upregulation to be expected, as in Parkinson's disease. A recent report has suggested that D2 upregulation is less persistent in Parkinson's disease that presents bilaterally (Wenning *et al.*, 1998), and it is interesting that extrapyramidal symptoms in DLB have been noted to be more symmetrical at presentation than in Parkinson's disease (Gnanalingham *et al.*, 1997). The low levels of D2 receptors in DLB are likely to be responsible for the adverse neuroleptic reactions seen in these patients, and may also explain the reduced benefit from L-dopa compared with Parkinson's disease. Lower numbers of D2 receptors are more likely to be completely blocked, producing parkinsonian signs, by neuroleptic administration, and DLB patients can show severe sensitivity to typical as well as recently developed neuroleptics, which have reduced propensity to induce extrapyramidal symptoms in other patient groups (McKeith *et al.*, 1995).

In Alzheimer's disease there was no change in D2 receptor binding or distribution compared with controls, consistent with the normal level of dopamine uptake site binding in the present report. This is in spite of neuroleptic medication in the majority of patients, which has been reported to increase D2 density in Alzheimer's disease and also in schizophrenia and in animal models (Seeman *et al.*, 1987). Alzheimer's disease patients seldom show sensitivity to neuroleptics (McKeith *et al.*, 1992).

D2 binding was found to be reduced with age in Parkinson's disease, with non-significant trends in other groups, consistent with the reported loss of D2 binding with increasing age previously reported in normal individuals over a wider age range *in vivo* (Volkow *et al.*, 1998), but in contrast to the lack of a decline with age *in vitro* (De Keyser *et al.*, 1990). Loss of D2 with age in normal cases may be slight and detectable only over a wide age range. Only in the DLB group was a gender difference apparent, males having lower D2 binding in the ventral striatum. This may relate to greater severity of extrapyramidal symptoms or to susceptibility to adverse neuroleptic reaction in males, and although this was not borne out in the present study it will be investigated in larger studies in DLB in future.

D1 receptor binding

There was no difference between disease groups in the density or pattern of binding, except for an increase in D1 binding in the caudal striatum in Parkinson's disease. Previously, D1 receptors have been found to be unaltered in Parkinson's disease (Mizukawa *et al.*, 1993; Piggott and Marshall, 1996), although there are some reports of a raised level of D1 receptors in Parkinson's disease untreated with L-dopa (Seeman *et al.*, 1987; Pearce *et al.*, 1990), and an elevation has been reported in one case of postencephalitic parkinsonism (Piggott and Marshall, 1996) and in narcolepsy (Aldrich *et al.*, 1992). D1 receptors are also elevated in MPTP experimental models of Parkinson's disease in cats and primates (Gnanalingham *et al.*, 1993; Graham *et al.*, 1993; Frohna *et al.*, 1995; Rioux *et al.*, 1997). The variation in reports of D1 binding density in Parkinson's disease may be due to the different coronal levels studied, since in the present work the elevation was only apparent caudally. L-Dopa therapy has been reported to increase D1 receptors in parkinsonian monkeys in the caudal striatum (Graham *et al.*, 1993; Rioux *et al.*, 1997), and raised D1 density might relate to the propensity for L-dopa medication to produce dyskinesias in long-term treated Parkinson's disease, in postencephalitic parkinsonism and in MPTP models (Graham *et al.*, 1993). Conversely, L-dopa therapy was reported to tend to decrease D1 binding in human *in vivo* imaging of Parkinson's disease (Turjanski *et al.*, 1997), which is consistent with the present finding of declining D1 receptors with disease duration in Parkinson's disease.

The present study confirms previous findings of no change in D1 binding in Alzheimer's disease (Cross *et al.*, 1984; Seeman *et al.*, 1987) or DLB (Piggott and Marshall, 1996). Reports of declining D1 receptor level with ageing have been conflicting. In the present study, D1 binding declined with age in the control and DLB groups, consistent with the decline previously reported (Rinne *et al.*, 1990) but in contrast to a report of no decline in D1 receptors with age (De Keyser *et al.*, 1990). It is probable that the age-related decline in D1 receptors is slight and reliably detected only over a long age range.

D3 receptor binding

The pattern of D3 binding is localized to limbic areas, particularly the nucleus accumbens, rather than motor areas of the striatum, with significant alterations in disease groups restricted to slightly elevated D3 binding in Alzheimer's disease in the dorsal striatum and somewhat reduced D3 binding in Parkinson's disease cases, with no deviation from the pattern of expression in DLB from controls. D3 receptors, while being structurally related to the D2-like dopamine receptor group, are localized to striosomes, like D1 receptors (Murray *et al.*, 1994), and may function in an inverse manner to D2 receptors (Levant, 1997). A previous report has shown no change in D3 binding in Parkinson's disease (Hurley *et al.*, 1996), while a recent investigation found reduced D3 receptors, particularly in the ventral striatum, in Parkinson's disease of more than 10 years' duration (Ryoo *et al.*, 1998). This latter finding is consistent with the lower D3 binding in Parkinson's disease in the present report, where there was an apparent 20% reduction in binding (although not statistically significant) in the nucleus accumbens, as well as the significant reduction in the caudal striatum. 6-Hydroxydopamine lesion decreased D3 receptors in rats (Lévesque *et al.*, 1995) and in MPTP monkeys (Morisette

et al., 1998). The present study compares D2 and D3 binding in the same cohort of patients (where only four cases have Parkinson's disease duration of more than 10 years) showing no upregulation of D3 receptors where D2 upregulation is demonstrated. Dopamine replacement by a dopamine agonist with D1 activity (but not by a D2-like agonist) has been reported to reverse experimentally reduced D3 receptors in the MPTP monkey (Morissette *et al.*, 1998). L-Dopa therapy, with presumed D1, D2 and D3 activity, might be expected to tend to reverse D3 receptor downregulation. Many typical neuroleptics are also D3 antagonists, and D3 binding or mRNA (in the olfactory tubercle) has been reported to be elevated in rats with neuroleptic administration (Wang *et al.*, 1996), but also not to have any effect (Lévesque *et al.*, 1995). The present study found slightly elevated D3 binding in Alzheimer's disease in the dorsal striatum.

Dopamine and homovanillic acid concentration

In the rostral striatum in DLB, greater loss occurred in the caudate compared with the putamen, and in the caudate the loss was uniform rostrocaudally. This implies different vulnerability of cell groups in the substantia nigra, and also suggests that there may be an influence of dopamine loss on non-motor characteristics of DLB, such as depression and reduced cognitive abilities (Gnanalingham *et al.*, 1997). It has been suggested that dopamine reductions of ~50–75% are required for the manifestation of extrapyramidal symptoms in Parkinson's disease (Bernheimer *et al.*, 1973; Hornykiewicz, 1973; Kish *et al.*, 1985), which is the degree of loss just reached in the DLB cases. Other reports of dopamine concentration in DLB have shown reductions at least as great as here (Langlais *et al.*, 1993; Ohara *et al.*, 1998). This should be sufficient explanation for the mild extrapyramidal symptoms in DLB. There would also seem to be sufficient reduction in dopamine concentration in the caudal putamen to have the potential to induce D2 upregulation in DLB as observed in Parkinson's disease. The HVA : dopamine ratio was increased greatly in Parkinson's disease, but in DLB the tendency to a higher ratio was not significantly raised over controls, even in the caudal putamen. DLB patients do not, therefore, appear to have the same capacity for presynaptic compensation for reduced dopamine as the remaining substantia nigra neurons do in Parkinson's disease and also in progressive supranuclear palsy (Bokobza *et al.*, 1984). This, combined with low level of postsynaptic D2 receptors, will probably result in greater motor deficits in DLB for the same amount of dopamine loss than in Parkinson's disease, which has both pre- and postsynaptic compensatory mechanisms.

The elevated dopamine concentration in the putamen in Alzheimer's disease may be a consequence of neuroleptic medication, or is perhaps some artefact of case selection. Previous reports have usually shown unchanged dopamine and HVA in Alzheimer's disease (Arai *et al.*, 1984; Langlais *et al.*, 1993), but decreased dopamine in the basal ganglia (Storga *et al.*, 1996; Ohara *et al.*, 1998) has also been found. There may be other factors contributing to movement abnormalities in Alzheimer's disease, involving nuclei beyond the nigrostriatal pathway, or synthesis and storage of dopamine rather than release (Marshall *et al.*, 1994). There was a greater range of HVA : dopamine ratio values in Alzheimer's disease than in DLB patients, especially caudally in the caudate, which may point to greater heterogeneity among the Alzheimer's disease patients. Neuroleptic administration tends to raise dopamine turnover in rats (Clow *et al.*, 1980; See *et al.*, 1992), and the raised HVA : dopamine ratio in Alzheimer's disease and DLB may arise partly in response to drug treatment.

Conclusion

Variations in the dopaminergic features which may underlie extrapyramidal symptoms in DLB, Parkinson's disease and Alzheimer's disease were revealed, with differences between DLB and Parkinson's disease presynaptically in dopamine loss and turnover, and dopamine uptake sites, as well as postsynaptically in D2 receptor density. DLB and Parkinson's disease both showed patterns of dopamine deficits which differed from those of controls and Alzheimer's disease patients. These findings are relevant to clinical practice since it is important to be able to distinguish Alzheimer's disease and DLB because prognosis and treatment strategies with neuroleptics, acetylcholinesterase inhibitors and L-dopa may need to vary. The caudal loss in dopamine uptake sites may be an effective way of separating DLB from Alzheimer's disease and Parkinson's disease *in vivo* with a suitable ligand, e.g. [^{123}I]β-CIT (β-carboxymethyl-iodophenyl-tropane), in combination with clinical diagnostic criteria, as reported by Donnemiller and colleagues (Donnemiller *et al.*, 1997). The lower expression caudally of D2 receptors in DLB compared with Alzheimer's disease, and especially the low D2 binding rostrally in DLB compared with Parkinson's disease, may also provide assistance in diagnosis by *in vivo* imaging of the D2 receptor in a way similar to that suggested for separating Parkinson's disease from parkinsonism-plus syndromes. Sequential imaging of dopamine uptake sites and D2 binding may also be valuable in monitoring the emergence of reduced L-dopa responsiveness and evaluating the effectiveness of therapeutic drugs in DLB.

References

Aldrich MS, Hollingsworth Z, Penney JB. Dopamine-receptor autoradiography of human narcoleptic brain. Neurology 1992; 42: 410–5.

Alexander GM, Schwartzman RJ, Brainard L, Gordon SW, Grothusen JR. Changes in brain catecholamines and dopamine uptake sites at different stages of MPTP parkinsonism in monkeys. Brain Res 1992; 588: 261–9.

Antonini A, Schwarz J, Oertel WH, Beer HF, Madeja UD, Leenders KL. [11C]raclopride and positron emission tomography in previously

untreated patients with Parkinson's disease: influence of L-dopa and lisuride therapy on striatal dopamine D2-receptors. Neurology 1994; 44: 1325–9.

Antonini A, Vontobel P, Psylla M, Gunther I, Maguire PR, Missimer J, et al. Complementary positron emission tomographic studies of the striatal dopaminergic system in Parkinson's disease. Arch Neurol 1995; 52: 1183–90.

Antonini A, Leenders KL, Vontobel P, Maguire RP, Missimer J, Psylla M, et al. Complementary PET studies of striatal neuronal function in the differential diagnosis between multiple system atrophy and Parkinson's disease. Brain 1997a; 120: 2187–95.

Antonini A, Schwarz J, Oertel WH, Pogarell O, Leenders KL. Long-term changes of striatal dopamine D2 receptors in patients with Parkinson's disease: a study with positron emission tomography and [11C]raclopride. Mov Disord 1997b; 12: 33–8.

Arai H, Kosaka K, Iizuka R. Changes of biogenic amines and their metabolites in postmortem brains from patients with Alzheimer-type dementia. J Neurochem 1984; 43: 388–93.

Ballard C, Grace J, McKeith I, Holmes C. Neuroleptic sensitivity in dementia with Lewy bodies and Alzheimer's disease [letter]. Lancet 1998a; 351: 1032–3.

Ballard CG, O'Brien J, Lowery K, Ayre GA, Harrison R, Perry R, et al. A prospective study of dementia with Lewy bodies. Age Ageing 1998b; 27: 631–6.

Bernheimer H, Birkmayer W, Hornykiewicz O, Jellinger K, Seitelberger F. Brain dopamine and the syndromes of Parkinson and Huntington. Clinical, morphological and neurochemical correlations. J Neurol Sci 1973; 20: 415–55.

Bokobza B, Ruberg M, Scatton B, Javoy-Agid F, Agid Y. [3H]spiperone binding, dopamine and HVA concentrations in Parkinson's disease and supranuclear palsy. Eur J Pharmacol 1984; 99: 167–75.

Booij J, Tissingh G, Boer GJ, Speelman JD, Stoof JC, Janssen AG, et al. [123I]FP-CIT SPECT shows a pronounced decline of striatal dopamine transporter labelling in early and advanced Parkinson's disease. J Neurol Neurosurg Psychiatry 1997; 62: 133–40.

Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. [Review]. Acta Neuropathol (Berl) 1991; 82: 239–59.

Brucke T, Asenbaum S, Pirker W, Djamshidian S, Wenger S, Wober C, et al. Measurement of the dopaminergic degeneration in Parkinson's disease with [123I] beta-CIT and SPECT. Correlation with clinical findings and comparison with multiple system atrophy and progressive supranuclear palsy. J Neural Transm Suppl 1997; 50: 9–24.

Clow A, Theodorou A, Jenner P, Marsden CD. Changes in rat striatal dopamine turnover and receptor activity during one years neuroleptic administration. Eur J Pharmacol 1980; 63: 135–44.

Cortes R, Gueye B, Pazos A, Probst A, Palacios JM. Dopamine receptors in human brain: autoradiographic distribution of D1 sites. Neuroscience 1989; 28: 263–73.

Cross RB. Demonstration of neurofibrillary tangles in paraffin sections: a quick and simple method using a modification of Palmgren's method. Med Lab Sci 1982; 39: 67–9.

Cross AJ, Crow TJ, Ferrier IN, Johnson JA, Markakis D. Striatal dopamine receptors in Alzheimer-type dementia. Neurosci Lett 1984; 52: 1–6.

Czudek C, Reynolds GP. [3H] GBR 12935 binding to the dopamine uptake site in post-mortem brain tissue in schizophrenia. J Neural Transm 1989; 77: 227–30.

De Keyser J, Ebinger G, Vauquelin G. Age-related changes in the human nigrostriatal dopaminergic system. Ann Neurol 1990; 27: 157–61.

Dean B, Pavey G, Opeskin K. [3H]raclopride binding to brain tissue from subjects with schizophrenia: methodological aspects. Neuropharmacology 1997; 36: 779–86.

Donnemiller E, Heilmann J, Wenning GK, Berger W, Decristoforo C, Moncayo R, et al. Brain perfusion scintigraphy with 99mTc-HMPAO or 99mTc-ECD and 123I-beta-CIT single-photon emission tomography in dementia of the Alzheimer-type and diffuse Lewy body disease. Eur J Nucl Med 1997; 24: 320–5.

Eising EG, Muller TT, Zander C, Kuhn W, Farahati J, Reiners C, et al. SPECT-evaluation of the monoamine uptake site ligand [^{123}I]91R0-2-beta-carbomethoxy-3-beta-(4-iodophenyl)-tropane([^{123}I]beta-CIT) in untreated patients with suspicion of Parkinson disease. J Investig Med 1997; 45: 448–52.

Ellis RJ, Caligiuri M, Galasko D, Thal LJ. Extrapyramidal motor signs in clinically diagnosed Alzheimer disease. [Review]. Alzheimer Dis Assoc Disord 1996; 10: 103–14.

Forstl H, Levy R, Burns A, Luthert P, Cairns N. Disproportionate loss of noradrenergic and cholinergic neurons as cause of depression in Alzheimer's disease—a hypothesis. Pharmacopsychiatry 1994; 27: 11–5.

Frohna PA, Rothblat DS, Joyce JN, Schneider JS. Alterations in dopamine uptake sites and D1 and D2 receptors in cats symptomatic for and recovered from experimental parkinsonism. Synapse 1995; 19: 46–55.

Galasko D, Katzman R, Salmon DP, Hansen L. Clinical and neuropathological findings in Lewy body dementias. Brain Cogn 1996; 31: 166–75.

Giobbe D, Castellano GC, Podio V. Dopamine D2 receptor imaging with SPECT using IBZM in 16 patients with Parkinson disease. Ital J Neurol Sci 1993; 14: 165–9.

Gnanalingham KK, Smith LA, Hunter AJ, Jenner P, Marsden CD. Alterations in striatal and extrastriatal D-1 and D-2 dopamine receptors in the MPTP-treated common marmoset: an autoradiographic study. Synapse 1993; 14: 184–94.

Gnanalingham KK, Byrne EJ, Thornton A, Sambrook MA, Bannister P. Motor and cognitive function in Lewy body dementia: comparison with Alzheimer's and Parkinson's diseases. J Neurol Neurosurg Psychiatry 1997; 62: 243–52.

Graham WC, Sambrook MA, Crossman AR. Differential effect of chronic dopaminergic treatment on dopamine D1 and D2 receptors in the monkey brain in MPTP-induced parkinsonism. Brain Res 1993; 602: 290–303.

Gurevich EV, Bordelon Y, Shapiro RM, Arnold SE, Gur RE, Joyce JN. Mesolimbic dopamine D3 receptors and use of antipsychotics

in patients with schizophrenia. A postmortem study. Arch Gen Psychiatry 1997; 54: 225–32.

Guttman M. Receptors in the basal ganglia. [Review]. Can J Neurol Sci 1987; 14 (3 Suppl): 395–401.

Guttman M, Burkholder J, Kish SJ, Hussey D, Wilson A, DaSilva J, et al. [11C]RTI-32 PET studies of the dopamine transporter in early dopa-naive Parkinson's disease: implications for the symptomatic threshold. Neurology 1997; 48: 1578–83.

Hall H, Sedvall G, Magnusson O, Kopp J, Halldin C, Farde L. Distribution of D1- and D2-dopamine receptors, and dopamine and its metabolites in the human brain. Neuropsychopharmacology 1994; 11: 245–56.

Herroelen L, De Backer JP, Wilczak N, Flamez A, Vauquelin G, De Keyser J. Autoradiographic distribution of D3-type dopamine receptors in human brain using [^{3}H]7-hydroxy-N,N-di-n-propyl-2-aminotetralin. Brain Res 1994; 648: 222–8.

Hierholzer J, Cordes M, Venz S, Schelosky L, Harisch C, Richter W, et al. Loss of dopamine-D2 receptor binding sites in parkinsonian plus syndromes. J Nucl Med 1998; 39: 954–60.

Hornykiewicz O. Dopamine in the basal ganglia. Its role and therapeutic implications (including the clinical use of L-DOPA). [Review]. Br Med Bull 1973; 29: 172–8.

Huang N, Ase AR, Hebert C, van Gelder NM, Reader TA. Effects of chronic neuroleptic treatments on dopamine D1 and D2 receptors: homogenate binding and autoradiographic studies. Neurochem Int 1997; 30: 277–90.

Hurd YL, Herkenham M. Molecular alterations in the neostriatum of human cocaine addicts. Synapse 1993; 13: 357–69.

Hurley MJ, Stubbs CM, Jenner P, Marsden CD. D3 receptor expression within the basal ganglia is not affected by Parkinson's disease. Neurosci Lett 1996; 214: 75–8.

Kalra S, Bergeron C, Lang AE. Lewy body disease and dementia. A review. [Review]. Arch Intern Med 1996; 156: 487–93.

Kazee AM, Cox C, Richfield EK. Substantia nigra lesions in Alzheimer disease and normal aging. Alzheimer Dis Assoc Disord 1995; 9: 61–7.

Kinnear PR, Gray CD. SPSS for Windows made simple. Hove: Lawrence Erlbaum; 1994.

Kish SJ, Chang LJ, Mirchandani L, Shannak K, Hornykiewicz O. Progressive supranuclear palsy: relationship between extrapyramidal disturbances, dementia, and brain neurotransmitter markers. Ann Neurol 1985; 18: 530–6.

Kish SJ, Shannak K, Hornykiewicz O. Uneven pattern of dopamine loss in the striatum of patients with idiopathic Parkinson's disease. Pathophysiologic and clinical implications. N Engl J Med 1988; 318: 876–80.

Kohler C, Radesater AC. Autoradiographic visualization of dopamine D-2 receptors in the monkey brain using the selective benzamide drug [3H]raclopride. Neurosci Lett 1986; 66: 85–90.

Konagaya M, Goto Y, Matsuoka Y, Konishi T, Konagaya Y. Neuroleptic malignant syndrome-like condition in multiple system atrophy. [letter]. J Neurol Neurosurg Psychiatry 1997; 63: 120–1.

Kopin IJ. The pharmacology of Parkinson's disease therapy: an update. [Review]. Annu Rev Pharmacol Toxicol 1993; 33: 467–95.

Landwehrmeyer B, Mengod G, Palacios JM. Dopamine D3 receptor mRNA and binding sites in human brain. Mol Brain Res Brain Res 1993; 18: 187–92.

Langlais PJ, Thal L, Hansen L, Galasko D, Alford M, Masliah E. Neurotransmitters in basal ganglia and cortex of Alzheimer's disease with and without Lewy bodies. Neurology 1993; 43: 1927–34.

Levant B. The D3 dopamine receptor: neurobiology and potential clinical relevance. [Review]. Pharmacol Rev 1997; 49: 231–52.

Lévesque D, Diaz J, Pilon C, Martres MP, Giros B, Souil E, et al. Identification, characterization, and localization of the dopamine D3 receptor in rat brain using 7-[^{3}H]hydroxy-N,N-di-n-propyl-2-aminotetralin. Proc Natl Acad Sci USA 1992; 89: 8155–9.

Lévesque D, Martres MP, Diaz J, Griffon N, Lammers CH, Sokoloff P, et al. A paradoxical regulation of the dopamine D3 receptor expression suggests the involvement of an anterograde factor from dopamine neurons. Proc Natl Acad Sci USA 1995; 92: 1719–23.

Liu Y, Stern Y, Chun MR, Jacobs DM, Yau P, Goldman JE. Pathological correlates of extrapyramidal signs in Alzheimer's disease. Ann Neurol 1997; 41: 368–74.

Lopez OL, Wisnieski SR, Becker JT, Boller F, DeKosky ST. Extrapyramidal signs in patients with probable Alzheimer disease. Arch Neurol 1997; 54: 969–75.

Love S, Wilcock GK, Matthews SM. No correlation between nigral degeneration and striatal plaques in Alzheimer's disease. Acta Neuropathol (Berl) 1996; 91: 432–6.

Malmberg A, Jackson DM, Eriksson A, Mohell N. Unique binding characteristics of antipsychotic agents interacting with human dopamine D2A, D2B, and D3 receptors. Mol Pharmacol 1993; 43: 749–54.

Marshall EF, Perry EK, Perry RH, McKeith IG, Fairbairn AF, Thompson P. Dopamine metabolism in post-mortem caudate nucleus in neurodegenerative disorders. Neurosci Res Commun 1994; 14: 17–25.

McKeith IG, Fairbairn A, Perry R, Thompson P, Perry E. Neuroleptic sensitivity in patients with senile dementia of Lewy body type [see comments]. Br Med J 1992; 305: 673–8. Comment in: Br Med J 1992; 305: 1158–9.

McKeith IG, Ballard CG, Harrison RW. Neuroleptic sensitivity to risperidone in Lewy body dementia [letter; comment]. Lancet 1995; 346: 699. Comment on: Lancet 1995; 346: 185.

McKeith IG, Galasko D, Kosaka K, Perry EK, Dickson DW, Hansen LA, et al. Consensus guidelines for the clinical and pathologic diagnosis of dementia with Lewy bodies (DLB): report of the consortium on DLB international workshop. [Review]. Neurology 1996; 47: 1113–24.

McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984; 34: 939–44.

Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. Neurology 1991; 41: 479–86.

Mizukawa K, McGeer EG, McGeer PL. Autoradiographic study on dopamine uptake sites and their correlation with dopamine levels and their striata from patients with Parkinson disease, Alzheimer disease, and neurologically normal controls. Mol Chem Neuropathol 1993; 18: 133–44.

Moratalla R, Quinn B, DeLanney LE, Irwin I, Langston JW, Graybiel AM. Differential vulnerability of primate caudate-putamen and striosome-matrix dopamine systems to the neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Proc Natl Acad Sci USA 1992; 89: 3859–63.

Morissette M, Goulet M, Grondin R, Blanchet P, Bedard PJ, Di Paolo T, et al. Associative and limbic regions of monkey striatum express high levels of dopamine D3 receptors: effects of MPTP and dopamine agonist replacement therapies. Eur J Neurosci 1998; 10: 2565–73.

Murray AM, Ryoo HL, Gurevich E, Joyce JN. Localization of dopamine D_3 receptors to mesolimbic and D_2 receptors to mesostriatal regions of human forebrain. Proc Natl Acad Sci USA 1994; 91: 11271–5.

Murray AM, Weihmueller FB, Marshall JF, Hurtig HI, Gottleib GL, Joyce JN. Damage to dopamine systems differs between Parkinson's disease and Alzheimer's disease with parkinsonism. Ann Neurol 1995; 37: 300–12.

Ohara K, Kondo N, Ohara K. Changes of monoamines in post-mortem brains from patients with diffuse Lewy body disease. Prog Neuropsychopharmacol Biol Psychiatry 1998; 22: 311–7.

Pearce RK, Seeman P, Jellinger K, Tourtellotte WW. Dopamine uptake sites and dopamine receptors in Parkinson's disease and schizophrenia. Eur Neurol 1990; 30 Suppl 1: 9–14.

Perry EK, Curtis M, Dick DJ, Candy JM, Atack JR, Bloxham CA, et al. Cholinergic correlates of cognitive impairment in Parkinson's disease: comparisons with Alzheimer's disease. J Neurol Neurosurg Psychiatry 1985; 48: 413–21.

Perry EK, Marshall E, Thompson P, McKeith IG, Collerton D, Fairbairn AF, et al. Monoaminergic activities in Lewy body dementia: relation to hallucinosis and extrapyramidal features. J Neural Transm Park Dis Dementia Sect 1993; 6: 167–77.

Perry E, Court J, Goodchild R, Griffiths M, Jaros E, Johnson M, et al. Clinical neurochemistry: developments in dementia research based on brain bank material. J Neural Transm 1998; 105: 915–33.

Perry RH, Irving D, Blessed G, Fairbairn A, Perry EK. Senile dementia of Lewy body type. A clinically and neuropathologically distinct form of Lewy body dementia in the elderly. J Neurol Sci 1990; 95: 119–39.

Perry R. A guide to the cortical regions. In: Roberts GW, Leigh PN, Weinberger DR, editors. Neuropsychiatric disorders. London: Wolfe; 1993. p. 1.1–1.10.

Perry RH, Jaros EB, Irving D, Scoones DJ, Brown A, McMeekin WM, et al. What is the neuropathological basis of dementia with Lewy bodies? In: Perry RH, McKeith IG, Perry EK, editors. Dementia with Lewy bodies: clinical, pathological, and treatment issues. Cambridge: Cambridge University Press; 1996. p. 212–23.

Piggott MA, Marshall EF. Neurochemical correlates of pathological and iatrogenic extrapyramidal symptoms. In: Perry RH, McKeith IG, Perry EK, editors. Dementia with Lewy bodies: clinical, pathological, and treatment issues. Cambridge: Cambridge University Press; 1996. p. 449–67.

Piggott MA, Perry EK, McKeith IG, Marshall E, Perry RH. Dopamine D2 receptors in demented patients with severe neuroleptic sensitivity [letter] [corrected] [published erratum appears in Lancet 1994; 343: 1170]. Lancet 1994; 343: 1044–5.

Piggott MA, Perry EK, Marshall EF, McKeith IG, Johnson M, Melrose HL, et al. Nigrostriatal dopaminergic activities in dementia with Lewy bodies in relation to neuroleptic sensitivity: comparisons with Parkinson's disease. Biol Psychiatry 1998; 44: 764–74.

Piggott MA, Marshall EF, Thomas N, Lloyd S, Court JA, Jaros E, et al. Dopaminergic activities in the human striatum: rostro-caudal gradients of uptake sites and of D_1 and D_2 but not of D_3 receptor binding or dopamine. Neuroscience 1999; 90: 433–45.

Pizzolato G, Chierichetti F, Fabbri M, Cagnin A, Dam M, Ferlin G, et al. Reduced striatal dopamine receptors in Alzheimer's disease: single photon emission tomography study with the D2 tracer [123I]-IBZM. Neurology 1996; 47: 1065–8.

Reader TA, Ase AR, Huang N, Hebert C, van Gelder NM. Neuroleptics and dopamine transporters. Neurochem Res 1998; 23: 73–80.

Reiche W, Grundmann M, Huber G. Dopamine (D2) receptor SPECT with 123I-iodobenzamide (IBZM) in diagnosis of Parkinson syndrome. [German]. Radiologe 1995; 35: 838–43.

Rinne JO, Lonnberg P, Marjamáki P. Age-dependent decline in human brain dopamine D1 and D2 receptors. Brain Res 1990; 508: 349–52.

Rinne JO, Laihinen A, Rinne UK, Nagren K, Bergman J, Ruotsalainen U. PET study on striatal dopamine D2 receptor changes during the progression of early Parkinson's disease. Mov Disord 1993; 8: 134–8.

Rinne JO, Sahlberg N, Ruottinen H, Nagren K, Lehikoinen P. Striatal uptake of the dopamine reuptake ligand [11C]beta-CFT is reduced in Alzheimer's disease assessed by positron emission tomography. Neurology 1998; 50: 152–6.

Rioux L, Frohna PA, Joyce JN, Schneider JS. The effects of chronic levodopa treatment on pre- and postsynaptic markers of dopaminergic function in striatum of parkinsonian monkeys. Mov Disord 1997; 12: 148–58.

Ryoo HL, Pierrotti D, Joyce JN. Dopamine D_3 receptor is decreased and D_2 receptor is elevated in the striatum of Parkinson's disease. Mov Disord 1998; 13: 788–97.

Sahlberg N, Marjamáki P, Rinne JO. Different pattern of reduction of striatal dopamine reuptake sites in Alzheimer's disease and ageing: a post mortem study with [3H]CFT. Neurobiol Aging 1998; 19 (Suppl 4S): S242.

Schroder J, Silvestri S, Bubeck B, Karr M, Demisch S, Scherrer S, et al. D2 dopamine receptor up-regulation, treatment response, neurological soft signs, and extrapyramidal side effects in

schizophrenia: a follow-up study with 123I-iodobenzamide single photon emission computed tomography in the drug-naive state and after neuroleptic treatment. Biol Psychiatry 1998; 43: 660–5.

See RE, Toga AW, Ellison G. Autoradiographic analysis of regional alterations in brain receptors following chronic administration and withdrawal of typical and atypical neuroleptics in rats. J Neural Transm Gen Sect 1990; 82: 93–109.

See RE, Chapman MA, Murray CE, Aravagiri M. Regional differences in chronic neuroleptic effects on extracellular dopamine activity. Brain Res Bull 1992; 29: 473–8.

Seeman P, Bzowej NH, Guan HC, Bergeron C, Reynolds GP, Bird ED, et al. Human brain D1 and D2 dopamine receptors in schizophrenia, Alzheimer's, Parkinson's, and Huntington's diseases. Neuropsychopharmacology 1987; 1: 5–15.

Singer HS, Hahn IH, Moran TH. Abnormal dopamine uptake sites in postmortem striatum from patients with Tourette's syndrome. Ann Neurol 1991; 30: 558–62.

Stern Y, Liu X, Albert M, Brandt J, Jacobs DM, Del Castillo-Castaneda C, et al. Modeling the influence of extrapyramidal signs on the progression of Alzheimer disease. Arch Neurol 1996; 53: 1121–6.

Storga D, Vrecko K, Birkmayer JG, Reibnegger G. Monoaminergic neurotransmitters, their precursors and metabolites in brains of Alzheimer patients. Neurosci Lett 1996; 203: 29–32.

Tatsch K, Schwarz J, Mozley PD, Linke R, Pogarell O, Oertel WH, et al. Relationship between clinical features of Parkinson's disease and presynaptic dopamine transporter binding assessed with [^{123}I] IPT and single-photon emission tomography. Eur J Nucl Med 1997; 24: 415–21.

Tissingh G, Booij J, Winogrodzka A, van Royen EA, Wolters EC. IBZM- and CIT-SPECT of the dopaminergic system in parkinsonism. J Neural Transm [Suppl] 1997; 50: 31–7.

Tissingh G, Bergmans P, Booij J, Winogrodzka A, van Royen EA, Stoof JC, et al. Drug-naive patients with Parkinson's disease in Hoehn and Yahr stages I and II show a bilateral decrease in striatal dopamine transporters as revealed by [123I]beta-CIT SPECT. J Neurol 1998; 245: 14–20.

Turjanski N, Lees AJ, Brooks DJ. In vivo studies on striatal dopamine D1 and D2 site binding in L-dopa-treated Parkinson's disease patients with and without dyskinesias. Neurology 1997; 49: 717–23.

Tyrrell PJ, Sawle GV, Ibanez V, Bloomfield PM, Leenders KL, Frackowiak RS, et al. Clinical and positron emission tomographic studies in the 'extrapyramidal syndrome' of dementia of the Alzheimer type. Arch Neurol 1990; 47: 1318–23.

Victoroff J, Zarow C, Mack WJ, Hsu E, Chui HC. Physical aggression is associated with preservation of substantia nigra pars compacta in Alzheimer disease. Arch Neurol 1996; 53: 428–34.

Volkow ND, Wang GJ, Fowler JS, Ding YS, Gur RC, Gatley J, et al. Parallel loss of presynaptic and postsynaptic dopamine markers in normal aging. Ann Neurol 1998; 44: 143–7.

Waddington JL, O'Boyle KM. The D1 dopamine receptor and the search for its functional role: from neurochemistry to behaviour. Rev Neurosci 1987; 1: 157–84.

Walker Z, Costa DC, Janssen AG, Walker RW, Livingstone G, Katona CL. Dementia with Lewy bodies: a study of post-synaptic dopaminergic receptors with iodine-123 iodobenzamide single-photon emission tomography. Eur J Nucl Med 1997; 24: 609–14.

Wang W, Hahn KH, Bishop JF, Gao DQ, Jose PA, Mouradian MM. Up-regulation of D3 dopamine receptor mRNA by neuroleptics. Synapse 1996; 23: 232–5.

Watts RL. The role of dopamine agonists in early Parkinson's disease. [Review]. Neurology 1997; 49 (1 Suppl 1): S34–S48.

Wenning GK, Donnemiller E, Granata R, Riccabona G, Poewe W. 123I-beta-CIT and 123I-IBZM-SPECT scanning in levodopa-naive Parkinson's disease. Mov Disord 1998; 13: 438–45.

Wilson JM, Levey AI, Rajput A, Ang L, Guttman M, Shannak K, et al. Differential changes in neurochemical markers of striatal dopamine nerve terminals in idiopathic Parkinson's disease. Neurology 1996; 47: 718–26.

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