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THE PATTERN OF NEURODEGENERATION IN HUNTINGTON'S DISEASE: A COMPARATIVE STUDY OF CANNABINOID, DOPAMINE, ADENOSINE AND $GABA_A$ RECEPTOR ALTERATIONS IN THE HUMAN BASAL GANGLIA IN HUNTINGTON'S DISEASE

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Abstract—In order to investigate the sequence and pattern of neurodegeneration in Huntington's disease, the distribution and density of cannabinoid CB_1, dopamine D_1 and D_2, adenosine A_{2a} and $GABA_A$ receptor changes were studied in the basal ganglia in early (grade 0), intermediate (grades 1, 2) and advanced (grade 3) neuropathological grades of Huntington's disease. The results showed a sequential pattern of receptor changes in the basal ganglia with increasing neuropathological grades of Huntington's disease. First, the very early stages of the disease (grade 0) were characterized by a major loss of cannabinoid CB_1, dopamine D_2 and adenosine A_{2a} receptor binding in the caudate nucleus, putamen and globus pallidus externus and an increase in $GABA_A$ receptor binding in the globus pallidus externus. Second, intermediate neuropathological grades (grades 1, 2) showed a further marked decrease of CB_1 receptor binding in the caudate nucleus and putamen; this was associated with a loss of D_1 receptors in the caudate nucleus and putamen and a loss of both CB_1 and D_1 receptors in the substantia nigra. Finally, advanced grades of Huntington's disease showed an almost total loss of CB_1 receptors and the further depletion of D_1 receptors in the caudate nucleus, putamen and globus pallidus internus, and an increase in $GABA_A$ receptor binding in the globus pallidus internus.

These findings suggest that there is a sequential but overlapping pattern of neurodegeneration of GABAergic striatal efferent projection neurons in increasing neuropathological grades of Huntington's disease. First, GABA/enkephalin striatopallidal neurons projecting to the globus pallidus externus are affected in the very early grades of the disease. Second, GABA/substance P striatonigral neurons projecting to the substantia nigra are involved at intermediate neuropathological grades. Finally, GABA/substance P striatopallidal neurons projecting to the globus pallidus internus are affected in the late grades of the disease. In addition, the finding that cannabinoid receptors are dramatically reduced in all regions of the basal ganglia in advance of other receptor changes in Huntington's disease suggests a possible role for cannabinoids in the progression of neurodegeneration in Huntington's disease. © 2000 IBRO. Published by Elsevier Science Ltd.

Key words: receptor changes, caudate nucleus, putamen, globus pallidus, substantia nigra.

Huntington's disease is characterized by an atrophy of the caudate nucleus and putamen.[27] Medium spiny GABAergic striatal projection neurons, the predominant neostriatal cell type, are particularly vulnerable in Huntington's disease,[27] while there is selective sparing of cholinergic interneurons,[18,32] and interneurons containing somatostatin, neuropeptide Y, and NADPH-diaphorase.[10,19]

Two populations of GABAergic striatal efferent neurons can be demonstrated based on their projection targets and neuropeptide content.[6,22,46,50] Striatal neurons projecting to the globus pallidus externus (GPe) are enriched in met-enkephalin (enk), whereas the striatal neurons projecting to the globus pallidus internus (GPi) and to the substantia nigra (SN) are enriched in substance P.[50] Recent studies have suggested a differential pattern of degeneration of these projection neurons in Huntington's disease, with GABA/enk-containing neurons projecting to the GPe and GABA/substance P-containing striatal neurons projecting to the SN being preferentially affected in pre-symptomatic cases and in early degenerative grades of Huntington's disease, with relative sparing of GABA/substance P-containing neurons projecting to GPi.[1,16,49] By late grades of Huntington's disease, all striatal projection neurons show extensive loss. In the present study the validity of this proposed pattern of neuronal degeneration in Huntington's disease has been investigated by studying changes in the binding of a range of neurotransmitter receptors, including the CB_1 cannabinoid receptor,[39] in the basal ganglia of Huntington's disease patients.

Receptor binding studies in the human and rat brains have demonstrated that cannabinoid receptors are presynaptically localized on striatonigral and striatopallidal terminals in the SN and globus pallidus.[25,30,37] These findings, together with the demonstration that D_1 receptors in the SN and GPi regions, and, D_2 and A_{2a} receptors in the GPe region[21,34,59] are presynaptically localized on striatal efferent terminals suggest the possibility that cannabinoid receptors are co-localized with these various types of receptors in the SN and globus pallidus. Also, the well defined co-localization of the cannabinoid CB_1, dopamine D_1, dopamine D_2 and adenosine A_{2a} receptors in the caudate nucleus and putamen has enabled us to compare and contrast the receptor changes in the early and late grades of Huntington's disease in order to provide further information on the sequence and pattern of neurodegeneration in Huntington's disease.

EXPERIMENTAL PROCEDURES

Tissue collection

The human brain tissue used in these studies was obtained from the

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Abbreviations: enk, enkephalin; FNZ, flunitrazepam; GPe, globus pallidus externus; GPi, globus pallidus internus; NADPH, reduced nicotinamide adenine dinucleotide phosphate; SN, substantia nigra.

New Zealand Neurological Foundation Human Brain Bank in the Department of Anatomy, University of Auckland and the study was approved by the University of Auckland Human Subjects Ethics Committee.

All control subjects had previously been in good health with no known history of neurological disease or drug treatment and all had died suddenly without the opportunity of receiving any form of medical treatment. For both control and Huntington's disease cases, the brains were removed to the Department of Anatomy, University of Auckland, immediately following autopsy. On arrival, tissue blocks were immediately selected from various regions of the basal ganglia. The tissue blocks were frozen on dry ice and stored at −80°C prior to subsequent autoradiographical processing as detailed below. The *post mortem* delay in each case is described as the time interval between death and the freezing of the tissue blocks.

The control tissue consisted of *post mortem* human brains obtained from six adult subjects (aged 21–81 years; average age 59 years; average *post mortem* delay 10 h; see Table 1 for details). The Huntington's disease tissue was obtained from 10 patients diagnosed with Huntington's disease, and graded according to the five point (0–4) neuropathological grading scale criteria of Vonsattel and colleagues[43,67] (two subjects were grade 0, three subjects grade 1, three subjects grade 2, and two subjects grade 3; see Table 2 for details). The subjects ranged in age from 56–87 years, average age 63 years; average *post mortem* delay 16 h.

Autoradiography

For these studies frozen blocks of unfixed tissue were mounted on to cryostat chucks and 16-μm sections were thaw mounted on to gelatine/chrome-alum-coated slides. Sections were stored at −80°C until labelled.

All autoradiographical techniques have been previously described.[11,14,25] For each ligand used, triplicate sections from relevant regions of each brain were labelled. In brief, cannabinoid CB_1 receptors were labelled at 2.5 nM with [^{3}H]CP55,940 (Dupont/NEN; specific activity, 125 Ci/mmol). The sections were incubated for 2 h at 37°C in 50 mM Tris–HCl buffer (pH 7.4) with 5% bovine serum albumin. The sections were then washed twice at 4°C in 50 mM Tris buffer with 1% bovine serum albumin for 2 h. Non-specific binding was determined by incubation in the presence of 10 μm CP55,940. Dopamine D_1 receptors were identified using 1 nM [^{3}H]SCH23390 (Dupont/NEN; specific activity, 80.4 Ci/mmol) in 50 mM Tris–HCl buffer (with 1 mM $MgCl_2$, 2 mM $CaCl_2$, 5 mM KCl, and 120 mM NaCl, pH 7.4); the sections were incubated for 30 min at room temperature before being rinsed twice for 5 min in ice-cold buffer. Non-specific binding was determined by incubation in the presence of 1 μM dopamine. Dopamine D_2 receptors were labelled for 20 min at room temperature in 3 nM [^{3}H]Raclopride (Dupont/NEN; specific activity, 79.5 Ci/mmol) in 170 mM Tris–HCl buffer (with 1 mM $MgCl_2$, 2 mM $CaCl_2$, 5 mM KCl, and 120 mM NaCl, pH 7.7); the sections were rinsed four times for 1 min each in ice-cold buffer. Non-specific binding was determined by incubation in the presence of 1 μM dopamine. Sections for adenosine A_{2a} binding were preincubated for 30 min in 1 U/ml adenosine deaminase (Sigma, type IV) in 50 mM Tris–HCl buffer (with 10 mM $MgCl_2$, pH 7.4), before labelling with 5 nM [^{3}H]CGS21680 (Dupont/NEN; specific activity, 42.6 Ci/mmol) for 2 h; the sections were then rinsed and washed twice for 5 min in buffer before being rinsed in ice-cold distilled H_20. Non-specific binding was determined by incubation in the presence of 20 μM 2-chloroadenosine. $GABA_A$ receptors were labelled using 1 nM [^{3}H]flunitrazepam (FNZ, Amersham; specific activity, 84 Ci/mmol) in 50 mM Tris–HCl buffer, pH 7.4; the sections were incubated for 1 h at 4°C and then washed twice for 1 min in ice-cold buffer before being rinsed in ice-cold distilled H_2O. Non-specific binding was determined by incubation in the presence of 1 μM FNZ. All sections were fan-dried at 4°C overnight and placed in X-ray cassettes with tritium-microscale calibration slides (Amersham), where they were exposed to tritium-sensitive hyperfilm for 10 weeks prior to developing. Integrative density measurements of each region were made using the MD30 Image Analysis System (Leading Edge Pty, Australia). The binding in the Huntington's disease brains is presented as a percentage of the mean of the binding measured in control brains. For Grade 1 and 3 the data are presented as the mean percentage difference ± S.E.M. For Grade 0 and 3, where there were only two cases, the mean percentage difference for each case were averaged and are presented with their errors.

Table 1. Source of control *post mortem* human brain tissue

Case	Sex	Age (years)	*Post mortem* delay (h)	Cause of death
H47	M	81	6.5	Subarachnoid haemorrhage
H78	F	48	11.5	Coronary artery disease
H79	M	75	11	Myocardial infarction
H80	M	72	10	Myocardial infarction
H81	M	55	12	Myocardial infarction
H82	M	21	8.5	Carbon monoxide poisoning

Table 2. Source of *post mortem* Huntington's disease brain tissue

Case	Sex	Age (years)	*Post mortem* delay (h)	HD grade	(CAG)*n* in IT15	Cause of death
HC46	M	59	2.5	0	16/43	Chronic obstructive respiratory disease
HC66	M	62	19	0	27/41	Pneumonia
HC55	M	87	20	1	14/42	Perforated duodenal ulcer
HC51	M	58	4.5	1	16/43	Pneumonia
HC53	M	56	14	1	17/43	Bowel obstruction
HC52	F	61	23	2	19/46	Myocardial infarction
HC57	M	58	29	2	18/46	Myocardial infarction
HC61	M	65	6	2	18/47	Pneumonia
HC58	M	64	19	3	18/44	Pneumonia
HC48	M	62	20	3	17/47	Septicemia

Abbreviations used in the figures and tables

CN	caudate nucleus
ENK	enkephalin
HD	Huntington's disease
PU	putamen
SNc	substantia nigra pars compacta
SNr	substantia nigra pars reticulata
SP	substance P
VS	ventral striatum

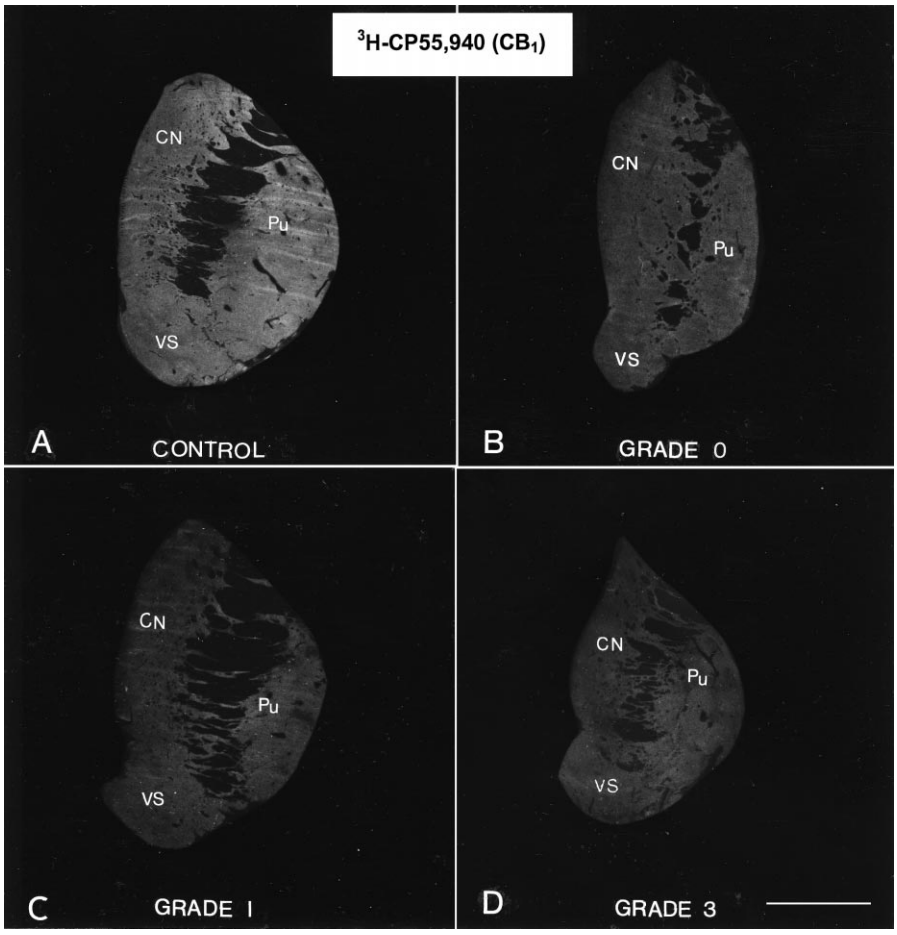

Fig. 1. Autoradiograms showing the binding of [^{3}H]CP55,940 to cannabinoid CB_1 receptors in the caudate nucleus and putamen of: (A) control; (B) grade 0 Huntington's disease; (C) grade 1 Huntington's disease; and (D) grade 3 Huntington's disease brains. There is a moderate decrease in CB_1 receptor binding at grade 0 (B) with a further marked loss of receptors at more advanced grades of Huntington's disease (C, D). Scale bar = 1 cm.

RESULTS

The principal aim of this study was to investigate the pattern of cannabinoid CB_1, dopamine D_1 and D_2, adenosine A_{2a} and $GABA_A$ receptor changes in the basal ganglia in the human brain in early (grade 0), intermediate (grade 1, 2) and late (grade 3) neuropathological grades of Huntington's disease in order to gain further information on the possible neuronal co-localization of these receptors in the human basal ganglia and on the sequence and pattern of neurodegeneration in Huntington's disease. The various receptors were demonstrated in the basal ganglia using receptor autoradiography following *in vitro* labelling of cryostat sections with tritiated ligands specific for the various receptor subtypes.

As shown in Figs 1–10, the pattern and density of autoradiographic receptor labelling for each of the receptors in the various nuclei of the basal ganglia—caudate nucleus, putamen, GPe, GPi and SN—were compared between control brains and early, intermediate and late stage Huntington's diseased brains. The density of the receptors in each of the nuclei in the basal ganglia was then determined using computerized densitometry methods (Tables 3–7). For all of the receptors studied the values observed in the control brains were comparable to previously reported values.[7,9,15,24,26,38,66] The results on the various types of receptors studied are detailed below.

Cannabinoid CB_1 receptors

The caudate nucleus and putamen, showed a moderately low level of cannabinoid CB_1 receptor binding in the normal brain (Figs 1A, 2A). As described previously,[24] careful examination of the pattern of receptor labelling in the caudate nucleus and putamen suggests a patchy distribution of receptors, especially in the caudal putamen at the level of the lenticular nucleus (Fig. 2A). The grade 0 Huntington's disease cases (Figs 1B, 2B) exhibited a moderate decrease in cannabinoid receptor binding (46–52%; Table 3) as compared to controls (Figs 1A, 2A). The cannabinoid receptor binding decreased dramatically in all Huntington's disease cases with more advanced pathology, that is, grade 1 and greater (Table 3). The grade 1 cases exhibited an average level of binding of only 21–31% of the normal (Figs 1C, 2C; Table 3), and further decreases were observed within the grade 2 and 3 cases, which exhibited binding similar to background levels.

Very high densities of cannabinoid receptor binding sites were seen in the globus pallidus of the control brains (Fig. 2A). The highest densities of receptors were present in the GPi and moderate densities of receptors were present throughout the rostrocaudal extent of the globus pallidus externus (Fig. 2A). Closer examination of the pattern of autoradiographic receptor labelling in the GPe revealed

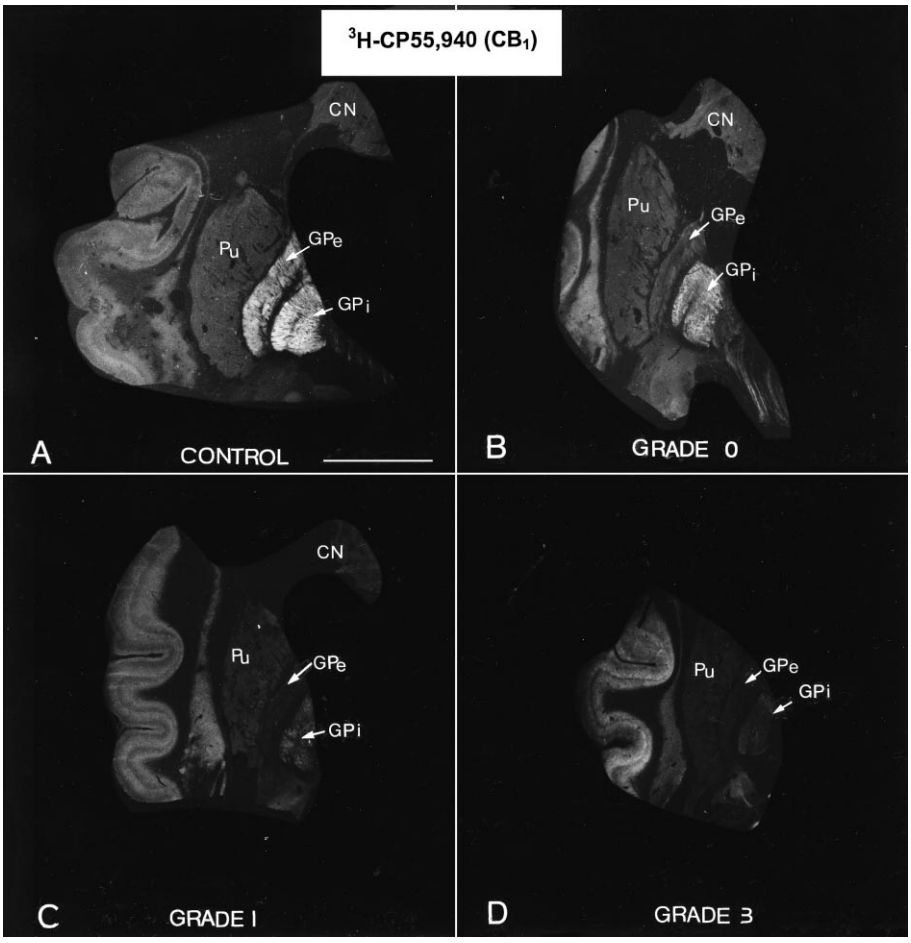

Fig. 2. Autoradiograms showing the binding of [^{3}H]CP55,940 to cannabinoid CB_1 receptors in the putamen and globus pallidus of the lenticular nucleus of: (A) control; (B) grade 0 Huntington's disease; (C) grade 1 Huntington's disease; and (D) grade 3 Huntington's disease brains. In the putamen there is an increasing loss of CB_1 receptor binding from grade 0 (B) to advanced grades (C, D) with an almost total loss of receptors at grade 3 (D). In the globus pallidus there is a differential loss of receptors with increasing neuropathological grades of Huntington's disease; CB_1 receptor binding is almost totally lost at grade 0 in the GPe (B), but is not totally lost in the GPi until grade 3 (D). Scale bar = 1 cm.

some regional variations in the density and pattern of receptor binding; higher density patches appeared to be present in some regions, with the highest density of labelling being present in the rostrolateral region of the complex and with lower densities of binding in the ventral pallidum.

Cannabinoid receptor binding was decreased dramatically in both pallidal segments in all cases of Huntington's disease (Fig. 2B–D). Within the very early stages of Huntington's disease (grade 0, Fig. 2B), the loss of CP55,940 binding was pronounced in the globus pallidus externus and density measurements showed that binding densities in GPe were reduced to 9% of normal (Table 3). In contrast, as shown in Table 3, the density of CB_1 binding in GPi had reduced to 19% of normal. However, in the more advanced cases of Huntington's disease (Fig. 2C–D), receptor binding in both segments had dramatically decreased to an average of between 3–7% of normal levels (Table 3).

Table 3. Cannabinoid CB_1 receptor levels in Huntington's disease brains—results are given as a percentage of the binding in control cases

HD grade	[^{3}H]CP55,940—% of control levels				
	CN	PU	GPe	GPi	SNr
0	46 ± 14	52 ± 17	9 ± 2	19 ± 4	19 ± 3
1	21 ± 4	31 ± 5	15 ± 1	14 ± 3	10 ± 7
2	9 ± 5	20 ± 6	3 ± 2	3 ± 1	9 ± 2
3	8 ± 3	8 ± 2	7 ± 3	4 ± 2	4 ± 2

As described previously,[24,25] cannabinoid receptor labelling within the SN was very dense and discreetly localized to the pars reticulata. As shown in Fig. 7A–C and Table 3, the levels of cannabinoid binding showed a marked decrease in grade 0 (19% of normal), and even greater decreases by grade 1 (10% of normal). By grade 2, binding was undetectable above background levels.

Dopamine D_1 and D_2 receptors

Within the caudate nucleus and putamen a fairly homogeneous distribution of dopamine D_1 (Figs 3A, 4A) and D2 (Figs 5A, 6A) receptors was observed in the control brains. At grade 0 Huntington's disease, normal levels of D_1 receptor binding were present in the caudate nucleus and putamen (Figs 3B, 4B; Table 4), while a major loss of D_2 receptor binding was observed (Figs 5B, 6B; Table 5, average of 40–44% of normal). In grade 1 cases, the density of D_2 receptors in the caudate nucleus and putamen had further reduced to 6–7% of normal (Figs 5C, 6C; Table 5) and D_1 receptors showed a moderate decrease to 54–56% of normal (Table 4,

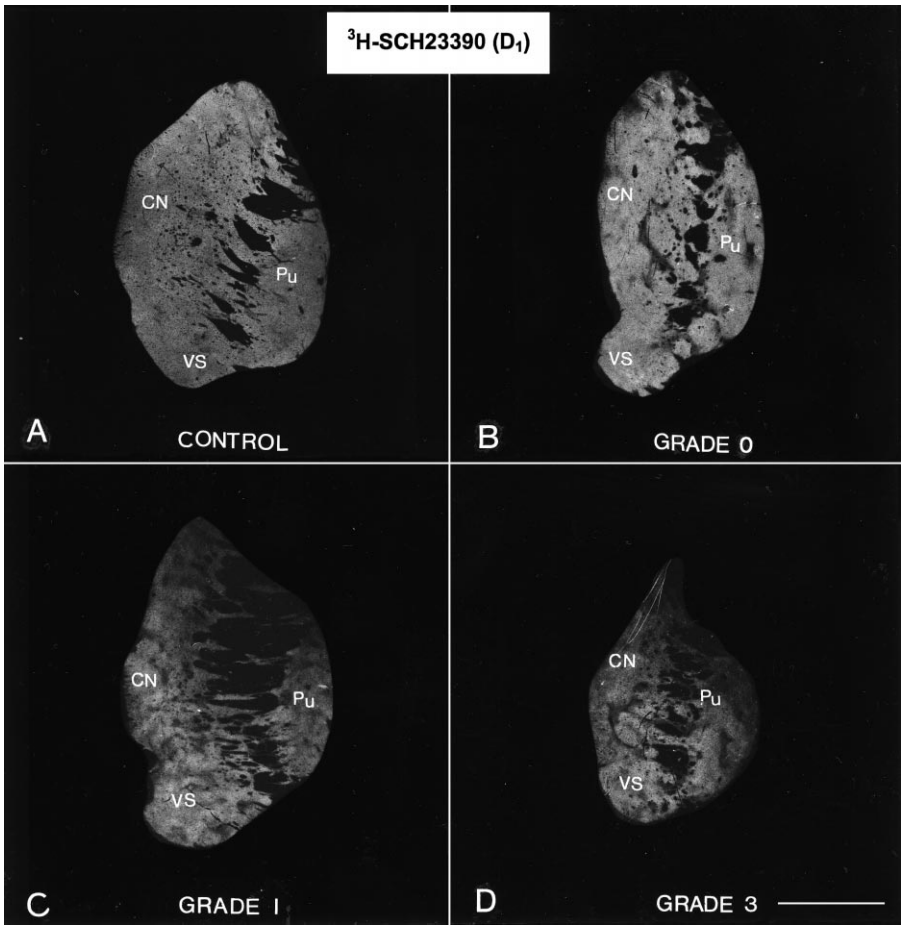

Fig. 3. Autoradiograms showing the binding of [^{3}H]SCH23390 to dopamine D_1 receptors in the caudate nucleus and putamen of: (A) control; (B) grade 0 Huntington's disease; (C) grade 1 Huntington's disease; and (D) grade 3 Huntington's disease brains. Grade 0 (B) showed generally normal levels of D_1 receptor binding but there was some evidence of a "patchy" loss of receptors in regions of the caudate nucleus and putamen. There was an increasing loss of D_1 receptor binding at more advanced grades of Huntington's disease with a further marked "patchy" loss of receptors (C, D). Scale bar = 1 cm.

Table 4. Dopamine D_1 receptor levels in Huntington's disease brains—results are given as a percentage of the binding in control cases

HD grade	[^{3}H]SCH23390—% of control levels				
	CN	PU	GPe	GPi	SNr
0	115 ± 19	118 ± 18	–	106 ± 7	74 ± 6
1	54 ± 20	56 ± 14	–	100 ± 3	80 ± 18
2	34 ± 7	28 ± 14	–	34 ± 20	31 ± 8
3	26 ± 9	32 ± 11	–	6 ± 2	11 ± 5

Table 5. Dopamine D_2 receptor levels in Huntington's disease brains—results are given as a percentage of the binding in control cases

HD grade	[^{3}H]Raclopride—% of control levels			
	CN	PU	GPe	GPi
0	44 ± 22	40 ± 21	6 ± 4	–
1	6 ± 2	7 ± 3	4 ± 4	–
2	21 ± 7	12 ± 4	1 ± 1	–
3	10 ± 1	10 ± 2	–	–

Figs 3C, 4C). In more advanced Huntington's cases (grades 2 and 3), the density of D_2 receptors in the caudate nucleus and putamen was barely above background levels (6–10% of normal; Figs 5D, 6D), and D_1 receptor densities further reduced to 26–34% of normal (Table 4; Figs 3D, 4D). Of particular interest was the finding that the loss of dopamine receptor binding within the caudate nucleus and putamen was not homogeneous. Irregularly shaped patches of both the caudate nucleus and putamen exhibited greater D_1 and D_2 binding loss than adjacent areas, giving the autoradiograms a "patchy" appearance (see Figs 3 and 5). This "patchy" pattern of receptor loss appeared reminiscent of the striosome/matrix compartmentation previously described for various neurochemical markers in the human caudate nucleus and putamen (see Ref. 28 for review). Furthermore, even in grade 3 Huntington's disease, a sub-population of D_1 receptors was preserved (Fig. 3D), while little D_2 binding was visible (Fig. 5D).

Within the normal globus pallidus, moderately low levels of D_1 receptors were located in GPi only (Fig. 4A), while moderate levels of D_2 receptors were present in the GPe (Fig. 6A). All Huntington's disease grades show a dramatic loss of D_2 receptor binding in GPe. In particular, dopamine D_2 receptors in the GPe show a dramatic reduction in the very early stages of Huntington's disease; in grade 0 brains D_2 receptor binding in GPe is reduced to 40–44% of controls, and, in grade 1 (reduced to 6–7% of control) and more advanced cases, D_2 labelling is barely above background levels (Fig. 6; Table 5). In contrast, the density of D_1 receptor binding in the GPi of grade 0 and grade 1 Huntington's

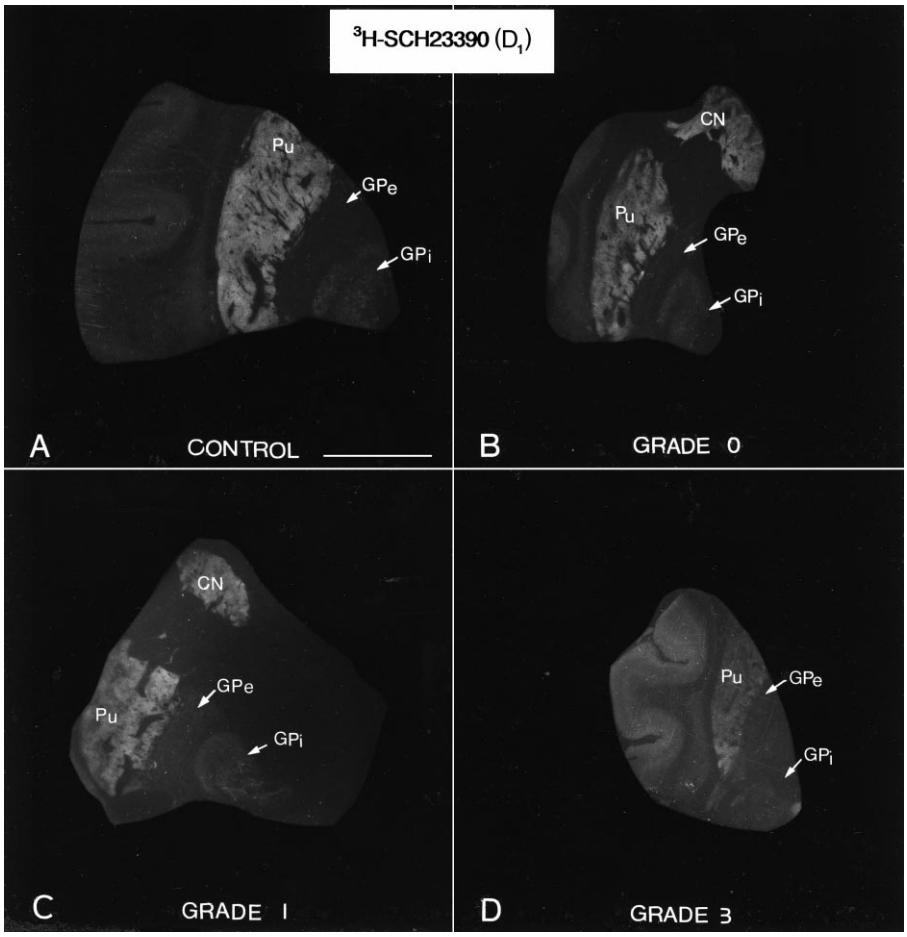

Fig. 4. Autoradiograms showing the binding of [^{3}H]SCH23390 to dopamine D_1 receptors in the putamen and globus pallidus of the lenticular nucleus of: (A) control; (B) grade 0 Huntington's disease; (C) grade 1 Huntington's disease; and (D) grade 3 Huntington's disease brains. In the putamen, there is an increasing "patchy" loss of D_1 receptor binding from grade 0 (B) to advanced grades (C, D). As in the control, there is an absence of D_1 receptors in the GPe at all neuropathological grades. In the GPi, D_1 receptor binding density at grades 0 (B) and 1 (C) is similar to the control, but is markedly reduced at grade 3 (D). Scale bar = 1 cm.

disease brains was equivalent to binding in the control pallidum (Table 4); intermediate levels of D_1 receptor binding were present at grade 2, while in the grade 3 cases, D_1 receptor binding was barely detectable (Fig. 4D; Table 4).

Within the SN only D_1 receptors were examined, as only very low levels of D_2 receptors were identified in the SN in the control brains. In normal control brains, D_1 receptors were discreetly localized within the pars reticulata of the SN (Fig. 7D). Only a slight loss of D_1 receptor binding was observed in grade 0 and grade 1 Huntington's disease (74–80% of control, Table 4; Fig. 7E, F). In the later grades of Huntington's disease the loss of receptor binding became more pronounced with D_1 receptor binding barely detectable above background levels in grade 3 Huntington's disease (Table 4).

Adenosine A_{2a} receptors

A_{2a} receptor binding was fairly homogeneous within the caudate nucleus and putamen of control brains (Figs 8A, 9A). Within the caudate nucleus and putamen a dramatic loss of adenosine A_{2a} receptor binding was observed in grade 0 Huntington's disease cases (34–35% of controls), and there was a further dramatic decrease in A_{2a} receptor binding in grade 1 Huntington's disease to 11–13% of controls (Figs 8C, 9C; Table 6); more advanced cases showed no detectable A_{2a} receptor binding (Figs 8D, 9D; Table 6). As for the dopamine receptors, the binding appeared to decline in a heterogeneous fashion, with irregularly shaped patches of receptors declining slightly more rapidly than the receptors in the surrounding regions (Fig. 8B, C).

In the globus pallidus, adenosine A_{2a} receptors were present only within the GPe (Fig. 9A). There was a dramatic and total loss of A_{2a} receptors from GPe in the very earliest stages of Huntington's disease; in all grade 0 cases and in all cases of more advanced pathology there was no detectable adenosine A_{2a} receptor binding (Fig. 9; Table 6).

$GABA_A$ receptors

$GABA_A$ receptor binding showed an increasing patchy loss in the caudate nucleus and putamen in grade 0 (Fig. 10B) and grade 1 (Fig. 10C) with an almost total loss of receptors in the caudate nucleus and putamen at more advanced grades of Huntington's disease (Fig. 10D). In contrast, $GABA_A$ receptor binding within the globus pallidus showed increased binding densities with increasing neuropathological grades of Huntington's disease (Fig. 10; Table 7). In confirmation of previous studies[16] a marked up-regulation of [^{3}H]FNZ binding was observed within the GPe in grade 0 (156% of control)

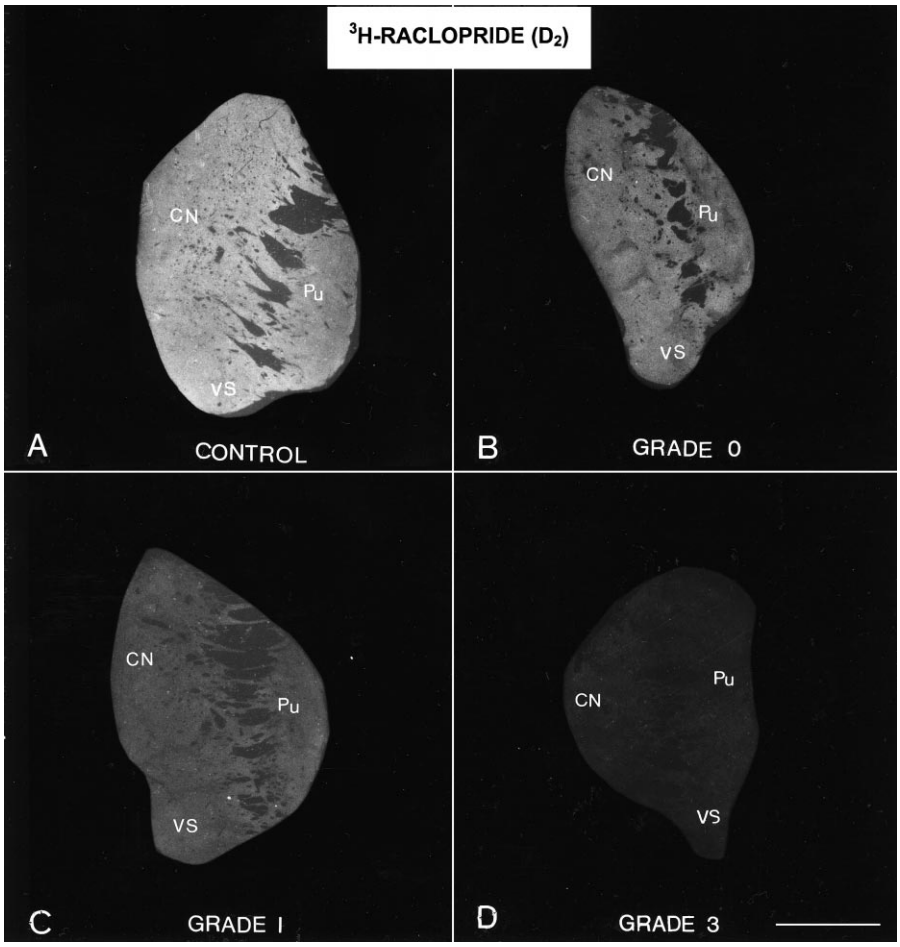

Fig. 5. Autoradiograms showing the binding of [^{3}H]Raclopride to dopamine D_2 receptors in the caudate nucleus and putamen of: (A) control; (B) grade 0 Huntington's disease; (C) grade 1 Huntington's disease; and (D) grade 3 Huntington's disease brains. There was a marked "patchy" decrease in D_2 receptor binding at grade 0 (B) with a further increasing loss of receptors at more advanced grades of Huntington's disease (C, D). Scale bar = 1 cm.

Table 6. Adenosine A_{2a} receptor levels in Huntington's disease brains—results are given as a percentage of the binding in control cases

HD grade	CGS21680 binding—% of control levels		
	CN	PU	GPe
0	34 ± 10	35 ± 1	2 ± 2
1	13 ± 7	11 ± 6	0
2	2 ± 0	0	1 ± 1
3	6 ± 1	1 ± 1	0

Table 7. $GABA_A$ receptor levels in Huntington's disease brains—results are given as a percentage of the binding in control cases

HD grade	[^{3}H]FNZ binding—% of control levels	
	GPe	GPi
0	156 ± 7	106 ± 4
1	129 ± 2	125 ± 5
2	126 ± 23	129 ± 7
3	139 ± 13	156 ± 10

and this was sustained in Huntington's disease cases with more advanced pathology (Fig. 10B–D; Table 7). Up-regulation of $GABA_A$ receptors within the GPi was not observed until grade 1; this up-regulation was sustained in grade 2 cases (129%) and further increased (156% of control) in more advanced grade 3 cases (Table 7).

DISCUSSION

It is now well established that medium spiny neurons of the caudate nucleus and putamen are preferentially vulnerable in Huntington's disease.[27] Furthermore, the subset of the medium spiny projection neurons containing GABA/enk demonstrate preferential dysfunction in terminal areas in the GPe.[49,52,61] In contrast, medium spiny neurons containing GABA/substance P projecting to the GPi are more resistant to dysfunction in early Huntington's disease. However, conflicting information on the relative loss of enkephalin-containing terminals versus substance P-containing terminals exists.[16,58,63] Since cannabinoid, dopamine (D_1 and D_2) and adenosine receptors are localized in various combinations on the cell bodies and terminal axons of striatal efferent neurons projecting to the GPe, GPi and SN (see Fig. 11A),[21,25,30,34,37,59] the present study has utilized the technique of receptor autoradiography to examine changes in cannabinoid, dopamine and adenosine receptors in the basal ganglia in Huntington's disease brains ranging from pathological grade 0 to grade 3 in order to further investigate the pattern of degeneration of striatal efferent neurons in this disease.

All receptors studied demonstrated a greater loss of binding

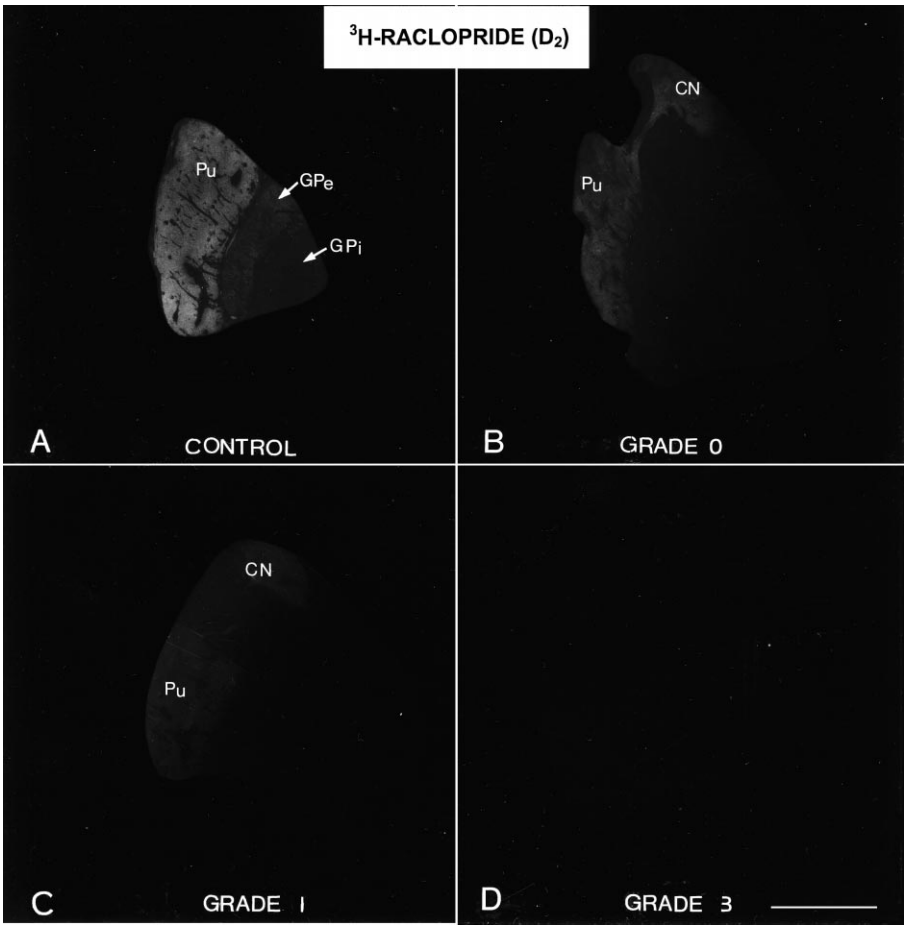

Fig. 6. Autoradiograms showing the binding of [^{3}H]raclopride to dopamine D_2 receptors in the putamen and globus pallidus of the lenticular nucleus of: (A) control; (B) grade 0 Huntington's disease; (C) grade 1 Huntington's disease; and (D) grade 3 Huntington's disease brains. In the putamen there is a very marked decrease in D_2 receptor binding at grade 0 (B) with a total loss of receptor binding at more advanced neuropathological grades of Huntington's disease (C, D). Compared with the control (A), there is a total loss of D_2 receptor binding in the GPe at grade 0 (B) and at more advanced grades (C, D), while the GPi shows no D_2 receptor binding in the control (A) and Huntington's disease (B–D) brains. Scale bar = 1 cm.

within the projection regions than within the caudate nucleus and putamen itself in early grade Huntington's disease, a finding consistent with a previous study by Richfield and Herkenham.[53] As suggested by these authors, two possible processes can explain this observation. First, it may represent perikaryal dysfunction associated with deficient production, processing or transport of receptors to terminals. Secondly, loss of receptors in the pallidum may reflect primary dysfunction in terminals followed by retrograde degeneration of projection neurons. Interestingly, presymptomatic cases demonstrate loss of enkephalin immunoreactivity in GPe, but preservation of enkephalin-containing neurons in the caudate nucleus and putamen, supporting primary terminal dysfunction.[52]

The results of this study indicate that the medium spiny neurons exhibit a selective vulnerability in early Huntington's disease. Figure 11 demonstrates the overall pattern of degeneration of the neurons, their terminals and the receptors within the basal ganglia as suggested by this study. The results show that, in agreement with previous studies, the medium spiny neurons in the caudate nucleus and putamen comprise of at least three different populations of GABAergic neurons: those containing enkephalin projecting to GPe; and two populations containing substance P, one projecting to the GPi and the other to the SN. While there may be some overlap within these populations, each group appears to have a different vulnerability to the disease process. Selective vulnerability was particularly indicated by the differential loss of dopamine D_1 and D_2 receptor binding. Binding to both of these receptors declined in a heterogeneous fashion from sub-populations of neurons, giving the autoradiograms a "patchy" appearance. The regions of binding were not discreet for D_1 and D_2 receptors but rather appeared to overlap in many regions. A similar finding was observed by Richfield *et al.*[54] in early Huntington's disease cases. What is particularly interesting to note in this study is the much more rapid loss of dopamine D_2 receptors as opposed to D_1 receptors, a finding which is contrary to earlier results.[54] Since D_2 receptors are believed to be localized predominantly on GABA/enk containing neurons which project to GPe, while D_1 receptors are localized to GABA/substance P-containing neurons projecting to GPi and SN pars reticulata, this finding therefore confirms previous studies of presymptomatic Huntington's disease allele carriers, where immunohistochemical results demonstrated that degeneration of striatal neurons projecting to GPe occurs earlier in the course of the disease than loss of neurons projecting to GPi,[1,49] a finding which has been further supported by other studies in early grade Huntington's disease.[16,58] Furthermore, the loss of dopamine D_1 receptor binding within the SN at grade 1, when levels within GPi

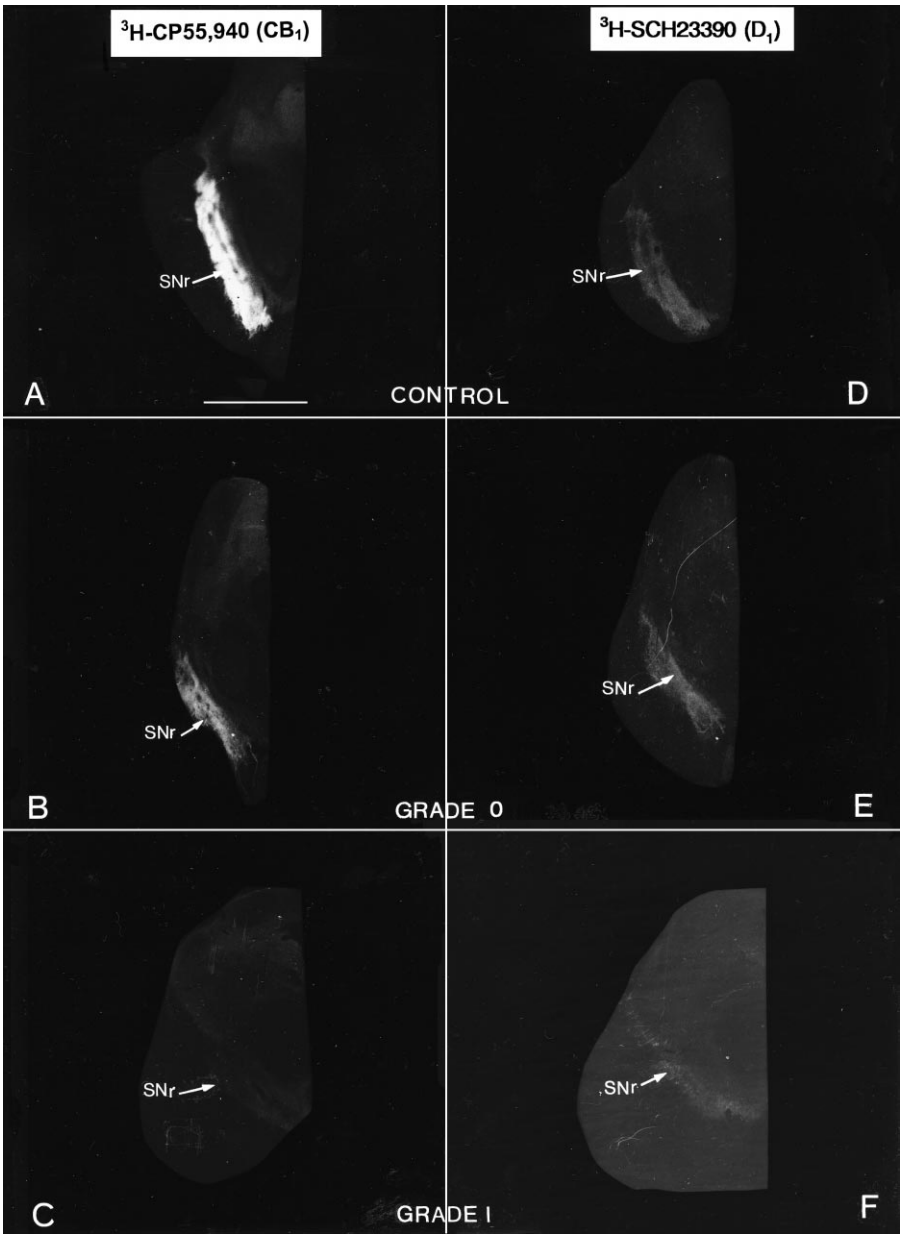

Fig. 7. Autoradiograms showing the distribution of cannabinoid CB_1 (A–C) and dopamine D_1 (D–F) receptors in the SN of control (A, D) and Huntington's disease (B, C, E, F) brains. The autoradiograms demonstrate the binding of [^{3}H]CP55,940 (A–C) to cannabinoid CB_1 receptors and [^{3}H]raclopride (D–F) to dopamine D_1 receptors in the SN in control (A, D); grade 0 Huntington's disease (B, E) and grade 1 Huntington's disease (C, F) brains. Cannabinoid CB_1 receptor binding in the SN shows very high densities in control brains (A); CB_1 receptor binding in the SN is reduced in grade 0 Huntington's disease (B) and is almost absent at higher neuropathological grades (C). Dopamine D_1 receptor binding in the SN shows no obvious change in grade 0 (E) compared with the control (D), but binding appears reduced in grade 1 (F) Huntington's disease cases. Scale bar = 1 cm.

were comparable to control levels, suggests that the population of GABA/substance P neurons projecting to SN pars reticulata, is distinct from the population of neurons projecting to GPi. Also, the heterogeneous patchy loss of D_1 and D_2 dopamine receptors (and adenosine A_{2a} and $GABA_A$ receptors) in the caudate nucleus and putamen in the earlier stages of the disease is in agreement with previous *in situ* and immunohistochemical studies by us[5,42] and others[29] suggesting that the projection neurons in the striosome compartment of the caudate nucleus and putamen may be especially vulnerable in early Huntington's disease.

Within the rat caudate nucleus and putamen it has been suggested that 15–20% of D_1 receptors are localized on non-medium spiny interneurons;[35] thus it may be that the surviving D_1 receptors present in the caudate nucleus and putamen in advanced diseased cases are localized on this subset of interneurons which are still present at Grade 3 Huntington's disease. This is in agreement with the results of our previous *in situ* studies on D_1 and D_2 receptor gene expression showing the relative survival of a subset of D_1 mRNA-positive neurons in the caudate nucleus and putamen of advanced Huntington's disease.[4] In contrast, the almost total loss of D_2 receptors (Figs 3, 4) and D_2 mRNA-expressing neurons[4] within the caudate nucleus and putamen in advanced Huntington's disease suggests that, unlike rat caudate nucleus and putamen,[3] a sub-population of D_2 receptors may not be present on the interneurons believed to be preserved in Huntington's disease.[18,32] However, in contrast to this study,

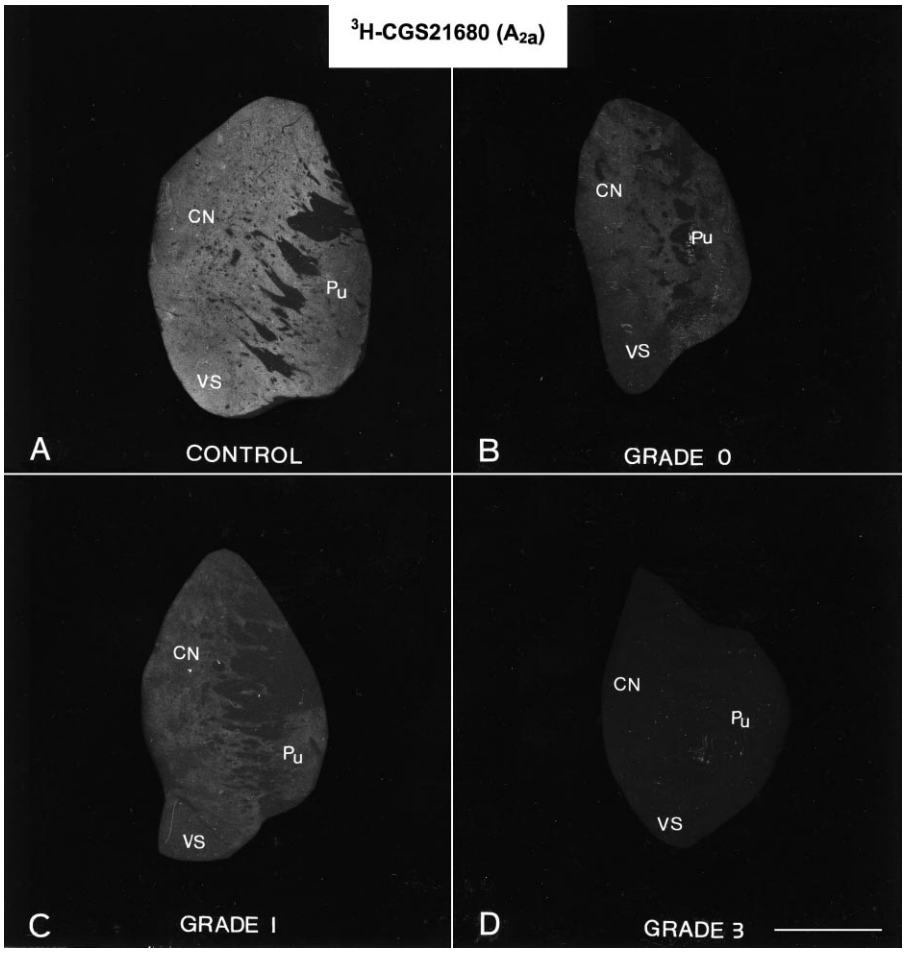

Fig. 8. Autoradiograms showing the binding of [^{3}H]CGS21680 to adenosine A_{2a} receptors in the caudate nucleus and putamen of: (A) control; (B) grade 0 Huntington's disease; (C) grade 1 Huntington's disease; and (D) grade 3 Huntington's disease brains. There is a very marked decrease in A_{2a} receptor binding in the caudate nucleus and putamen at grade 0 (B) with an almost total loss of receptors at more advanced grades of Huntington's disease (C, D). Scale bar = 1 cm.

a previous study,[53] demonstrated 30–40% of normal levels of D_2 receptors in grade 3 Huntington's disease, supporting the localization of D_2 receptors presynaptically on nigrostriatal terminals and on interneurons; the reasons for these differences are not clear. The almost total loss of D_1 receptor binding within the SN in grade 3 Huntington's disease confirms previous findings showing that D_1 receptors within the SN are localized exclusively to the terminals of striatal projection neurons.[66]

The results in this study confirm an earlier study by Martinez-Mir *et al.*[38] demonstrating A_{2a} receptor loss in the caudate nucleus and putamen in Huntington's disease. The loss of A_{2a} receptors in the caudate nucleus and putamen in the present study paralleled the loss of D_2 receptors. The similarities in the changes in A_{2a} and D_2 receptor binding was expected, as *in situ* hybridization studies have demonstrated that rat A_{2a} adenosine receptors are co-expressed in the same striatal neurons as D_2 dopamine receptors, with no A_{2a} receptors co-expressed with either D_1 receptors or substance P.[21,59] The loss of A_{2a} receptors from both GPe and the caudate nucleus and putamen in grade 0 again confirms the loss of this subset of medium spiny projection neurons early in the disease process. Studies in *post mortem* human brain have previously suggested that the A_{2a} site may be present on cholinergic interneurons within the caudate nucleus and putamen[33] and since the cholinergic interneurons are relatively spared in Huntington's disease,[18,32] then a proportion of A_{2a} receptors would be expected to be preserved in the caudate nucleus and putamen of Huntington's disease brains. However, a virtually total loss of A_{2a} binding was observed in the caudate nucleus and putamen in grades 1–3 Huntington's disease suggesting that the A_{2a} receptors are either localized solely to the medium spiny neurons, or that A_{2a} receptors are localized in part on cholinergic interneurons, and that these neurons are also vulnerable in early Huntington's disease.

Recent studies have demonstrated that adenosine A_{2a} receptors inhibit the activity of striatal dopamine D_2 receptors by decreasing their affinity for agonists[20] and by regulating their gene expression in enkephalinergic neurons.[60] A study by Popoli *et al.*[48] demonstrated that CGS21680 exhibits a protective effect on dopamine induced hyperactivity in the quinolinic acid-lesioned rat. The authors of this study therefore suggested that A_{2a} receptor agonists may be beneficial in the treatment of Huntington's disease. In support of this suggestion, studies have shown that the activation of A_{2a} receptors can enhance the electrically stimulated release of GABA in the pallidum.[40] However, loss of receptor binding in this area may limit the effectiveness of A_{2a} specific drugs, and furthermore, may be a contributing factor to the disease symptoms.

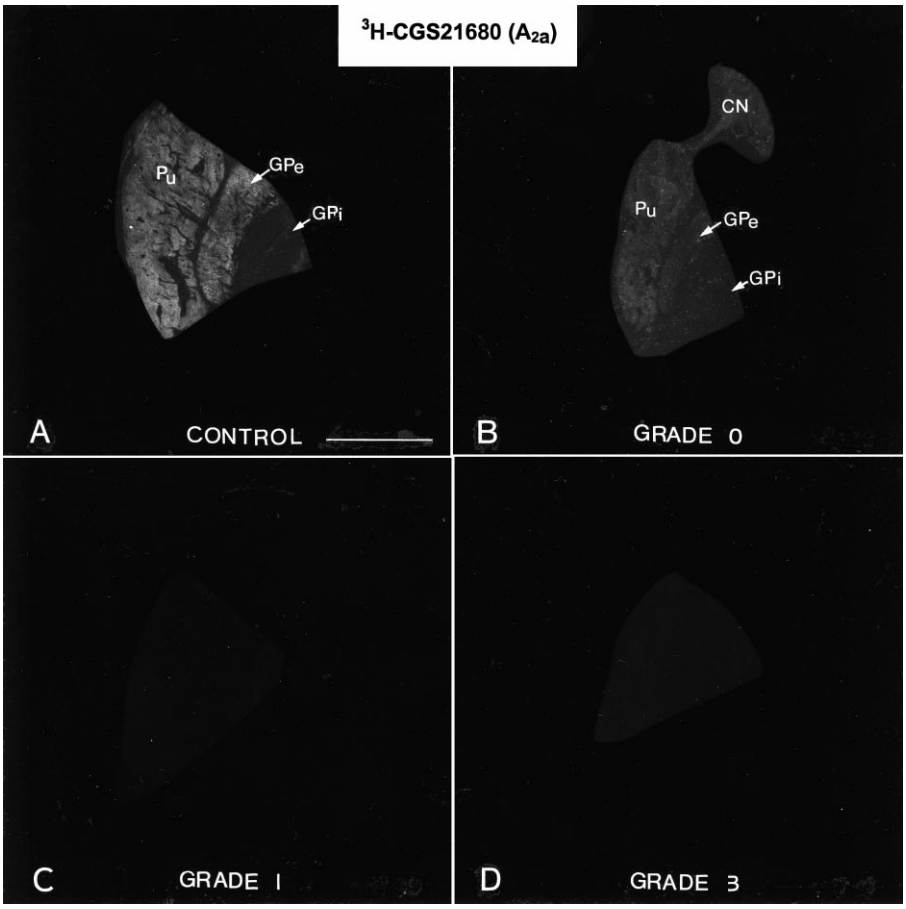

Fig. 9. Autoradiograms showing the binding of [^{3}H]CGS21680 to adenosine A_{2a} receptors in the putamen and globus pallidus of the lenticular nucleus of: (A) control; (B) grade 0 Huntington's disease; (C) grade 1 Huntington's disease; and (D) grade 3 Huntington's disease brains. In the putamen, there is a very marked decrease in A_{2a} receptor binding at grade 0 (B) with a total loss of receptors at more advanced grades of Huntington's disease (C, D). Compared with the control (A), there is a total loss of A_{2a} receptor binding in the GPe at grade 0 (B) and at more advanced grades (C, D), while the GPi shows no A_{2a} receptor binding in the control (A) and Huntington's disease (B–D) brains. Scale bar = 1 cm.

Within the caudate nucleus and putamen the loss of cannabinoid receptors was in between the loss of D_1 and D_2 receptors in grade 0 Huntington's disease, suggesting that cannabinoid receptors are localized on both GABA/enk and GABA/substance P projection neurons, as has been demonstrated previously.[37] Within the globus pallidus, cannabinoid receptor binding was dramatically decreased in the GPe in the very early Huntington's disease cases, and exceeded the loss of binding density within the GPi; this finding is consistent with GABA/enk neurons projecting to the GPe being more vulnerable in early Huntington's disease than GABA/substance P neurons projecting to the GPi. The selective vulnerability of striatal-GPe projection neurons is further supported by the finding that the loss of cannabinoid receptor binding within the GPe in grade 0 Huntington's disease is accompanied by a comparable loss of D_2 and A_{2a} receptor binding in the GPe. These findings therefore suggest that striatopallidal projection terminals in GPe degenerate at early stages of Huntington's disease. This pattern of degeneration is further supported by the observed up-regulation of GABA receptors in GPe in the grade 0 cases. These receptors are postsynaptic in the globus pallidus, and their up-regulation in Huntington's disease has been interpreted as a denervation supersensitivity phenomenon reflecting the loss of GABA input secondary to the degeneration of striatal neurons.[12,16,17,47,51]

In contrast to the receptor changes in the GPe, where cannabinoid and dopamine D_2 receptors were lost simultaneously in Huntington's disease, in the GPi the cannabinoid receptor changes preceded alterations in D_1 receptor binding. In grade 0 Huntington's disease there was a substantial loss of cannabinoid receptor binding in the GPi. However, in these cases D_1 receptor binding was normal and there was no evidence of up-regulation of $GABA_A$ receptors suggesting the preservation of the GPi synaptic terminals in these cases. Thus, in the grade 0 cases there appears to be a preferential loss of cannabinoid receptor binding in GPi prior to terminal degeneration. In grade 1 Huntington's disease, the findings are more complicated. A further decrease in cannabinoid receptor binding is observed, while the density of D_1 receptors remained at normal levels, suggesting intact terminals. The preservation of striatopallidal terminals is further supported by normal substance P concentrations in GPi in Grade 1 cases.[1,16,49] However, an up-regulation of $GABA_A$ receptors is detectable in grade 1 Huntington's disease, suggesting that alterations in the functioning of the medium spiny neurons, in the form of decreased GABA levels, are occurring prior to any detectable terminal degeneration.

Consistent with the results in the GPi is the finding of a similar pattern of changes in the SN. Thus, in grade 0 Huntington's disease cannabinoid receptors in the SN demonstrated a pronounced decrease in binding density

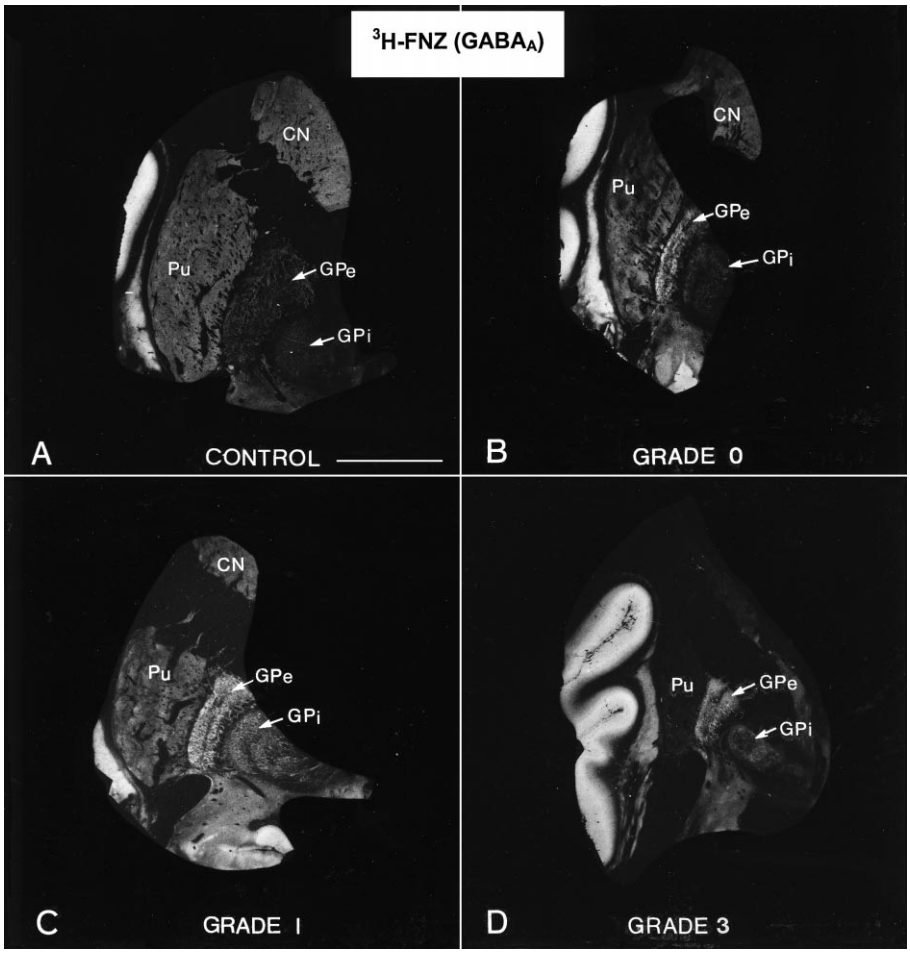

Fig. 10. Autoradiograms showing the binding of [^{3}H]FNZ to GABA$_A$ receptors in the putamen and globus pallidus of the lenticular nucleus of: (A) control; (B) grade 0 Huntington's disease; (C) grade 1 Huntington's disease; and (D) grade 3 Huntington's disease brains. There is a gradual increasing "patchy" loss of GABA$_A$ receptor binding in the putamen at grade 0 (B) and grade 1 (C) with an almost total loss of receptors at advanced grades of Huntington's disease (D). In the globus pallidus, there is a marked increase in GABA$_A$ receptor binding in the GPe at grade 0 and in both the GPe and GPi at more advanced grades of Huntington's disease (C, D). Scale bar = 1 cm.

but D_1 receptor binding was equivalent to that seen in controls. If D_1 receptors can be considered to be markers for striatonigral terminals then these findings would again suggest that the cannabinoid receptor binding is being compromised prior to the degeneration of the terminals. It is difficult to explain the possible functional significance of the loss of CB_1 receptors prior to the loss of co-localized dopamine receptors. In recent years several excellent studies have investigated the interactions of cannabinoid and dopamine in the projection nuclei of the basal ganglia[23,55–57] demonstrating a highly complex interaction between these two systems. It is interesting to speculate that perhaps the early down-regulation of cannabinoid receptors is a compensatory mechanism in Huntington's disease. Albin *et al.*[2] proposed a model for the early symptoms of Huntington's disease which demonstrates that decreased GABA/enk input to the GPe of the basal ganglia results in increased inhibition of the subthalamic nucleus, which in turn results in disinhibition of thalamocortical fibres. Several studies have suggested that cannabinoid receptor activation may inhibit the release of GABA from projection terminals,[41,64] thus loss of cannabinoid receptors may result in increased GABA release within these regions, which may compensate for the initial loss of GABAergic functioning. That alterations in cannabinoid receptor levels may significantly alter other neurochemistry was clearly demonstrated recently in the production of a mouse lacking cannabinoid receptors;[62] these animals demonstrated increases in substance P, dynorphin and enkephalin in the caudate nucleus and putamen.

Alternatively, while D_1 and cannabinoid receptors are clearly co-localized on striatonigral and striatopallidal projection terminals, it is possible that they display an uneven distribution on these terminals. This study would therefore imply that medium spiny neurons with a higher ratio of cannabinoid to D_1 receptors are preferentially degenerating in early Huntington's disease. Cannabinoid compounds such as the non-psychotropic HU-211 have been demonstrated to be neuroprotective;[13,44,68] however these compounds do not activate the CB_1 receptor. A recent study demonstrated that tetrahydrocannabinol exposure can lead to cell death via the CB_1 receptor;[8] high levels of cannabinoid receptors may therefore render the cells more sensitive if the disease process has resulted in increased levels of endogenous cannabinoid agonist as has been recently reported for schizophrenia.[36] Furthermore, any increase in endogenous agonist level could result in a down-regulation of CB_1 receptors. A down-regulation in cannabinoid receptors in response to chronic exposure to cannabinoids has been demonstrated previously.[45] We are currently investigating the levels of

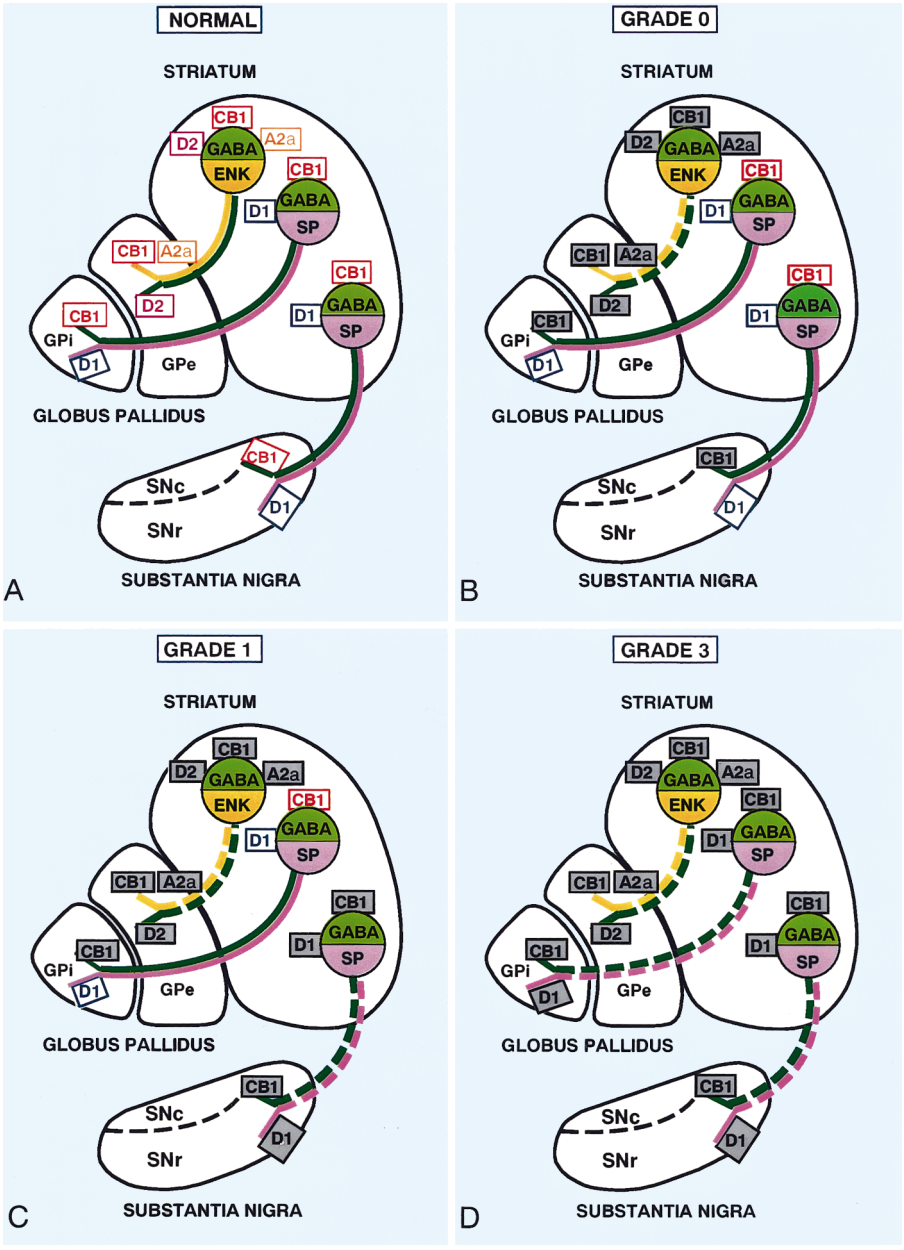

Fig. 11. Schematic summary diagrams demonstrating the relationship of the alterations in receptor binding to the pattern of degeneration of neurons in the basal ganglia in Huntington's disease. (A) demonstrates the neuronal localization of receptors in the normal human brain based on previous studies in animal and human brains. The findings presented in this study suggest that there are at least three sub-populations of GABAergic medium-sized spiny striatal efferent neurons: GABA/ENK neurons projecting to the GPe; and two populations of GABA/SP neurons projecting to either the GPi or the SNr. (B) summarizes the interpretation of the findings in grade 0 Huntington's disease cases; the early degeneration of GABA/ENK neurons projecting to the GPe is suggested by the loss of receptor binding both within the caudate nucleus and putamen and in the GPe. Furthermore, the selective loss of cannabinoid receptor binding at grade 0 in both the GPi and the SNr in the presence of normal D_1 receptors suggests that these terminals are still intact. (C) demonstrates the findings in grade 1 Huntington's disease: the degeneration of GABA/SP neurons projecting to the SNr is indicated by the loss of both cannabinoid and D_1 receptor binding in the caudate nucleus and putamen and the SNr; also, of note is the further loss of cannabinoid receptors within the GPi, without the loss of D_1 receptors in this region, suggesting that the terminals are still intact in the GPi. (D) demonstrates that the results of the binding studies suggest that by grade 3 Huntington's disease all pathways show advanced degeneration. The receptors lost in the various grades of Huntington's disease are outlined in black in B, C and D.

the endogenous agonists anandamide and 2-arachidonyl glycerol in these brains. Interestingly, a recent study demonstrated an increase in anandamide levels in the globus pallidus of reserpine-treated rats, which is a model of Parkinson's disease.[31]

Whether these various neurochemical changes are occurring in response to the disease process or are contributing to it is unclear. It is not yet possible to further elucidate the mechanisms involved here until the function of the Huntington's disease gene,[65] and the endogenous cannabinoid ligands are better understood. While the mechanism and significance of the cannabinoid receptor loss is speculative at present, this study suggests that selective vulnerability does exist among medium spiny neurons to the degenerative processes in Huntington's disease. Furthermore, this study emphasizes that the degeneration of terminals and receptors are not necessarily parallel processes. The findings here demonstrate the novel finding that cannabinoid receptor binding declines

dramatically in early grade Huntington's disease, prior to the apparent degeneration of the terminals as indicated by co-localized receptors. These changes may indicate that cannabinoids have a central role in the progression of neurodegeneration in Huntington's disease.

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REFERENCES

1. Albin R. L., Reiner A., Anderson K. D., Dure L. S., Handelin B., Balfour R., Whetsell W. O. J., Penney J. B. and Young A. B. (1992) Preferential loss of striato-external pallidal projection neurons in presymptomatic Huntington's disease. *Ann. Neurol.* **31,** 425–430.
2. Albin R. L., Young A. B. and Penney J. B. (1989) The functional anatomy of basal ganglia disorders. *Trends Neurosci.* **12,** 366–375.
3. Aubry J. M., Schulz M. F., Pagliusi S., Schulz P. and Kiss J. Z. (1993) Co-expression of dopamine D2 and substance P (neurokinin-1) receptor messenger RNAs by a sub-population of cholinergic neurons in the rat caudate nucleus and putamen. *Neuroscience* **53,** 417–424.
4. Augood S. J., Faull R. L. M. and Emson P. C. (1997) Dopamine D1 and D2 receptor gene expression in Huntington's disease caudate nucleus and putamen. *Ann. Neurol.* **42,** 215–221.
5. Augood S. J., Faull R. L. M., Love D. R. and Emson P. C. (1996) Reduction in enkephalin and substance P messenger RNA in the caudate nucleus and putamen of early grade Huntington's disease: a detailed cellular *in situ* hybridization study. *Neuroscience* **72,** 1023–1036.
6. Beckstead R. M. and Cruz C. J. (1986) Striatal axons to the globus pallidus entopenduncular nucleus and substantia nigra come mainly from separate cell populations in cat. *Neuroscience* **19,** 147–158.
7. Camps M., Cortes R., Gueye B., Probst A. and Palacios J. M. (1989) Dopamine-receptors in human brain—autoradiographic distribution of D2 sites. *Neuroscience* **28,** 275–290.
8. Chan G. C. K., Hinds T. R., Impey S. and Storm D. R. (1998) Hippocampal neurotoxicity of delta(9)-tetrahydrocannabinol. *J. Neurosci.* **18,** 5322–5332.
9. Cortes R., Gueye B., Pazos A., Probst A. and Palacios J. M. (1989) Dopamine-receptors in human brain—autoradiographic distribution of D1 sites. *Neuroscience* **28,** 263–273.
10. Dawbarn D., De Quidt M. E. and Emson P. C. (1985) Survival of basal ganglia neuropeptide-Y somatostatin neurons in Huntington's disease. *Brain Res.* **340,** 251–260.
11. Dragunow M., Tse C., Glass M. and Lawlor P. A. (1994) C-fos antisense inhibits basal expression of Krox 24 in rat caudate and neocortex. *Cell. molec. Neurobiol.* **14,** 395–405.
12. Enna S. J., Bennett J. P., Bylund D. B., Snyder S. H., Bird E. D. and Iversen L. L. (1976) Alterations of brain neurotransmitter binding in Huntington's chorea. *Brain Res.* **16,** 531–537.
13. Eshhar N., Striem S. and Biegon A. (1993) HU-211, a non-psychotropic cannabinoid, rescues cortical-neurons from excitatory amino-acid toxicity in culture. *NeuroReport* **5,** 237–240.
14. Faull R. L. M. and Villiger J. W. (1986) Benzodiazepine receptors in the human spinal cord: a detailed anatomical and pharmacological study. *Neuroscience* **17,** 791–802.
15. Faull R. L. M. and Villiger J. W. (1988) Multiple benzodiazepine receptors in the human basal ganglia: a detailed pharmacological and anatomical study. *Neuroscience* **24,** 433–451.
16. Faull R. L. M., Waldvogel H. J., Nicholson L. F. B. and Synek B. J. L. (1993) The distribution of GABAa-benzodiazepine receptors in the basal ganglia in Huntington's disease and in the quinolinic acid lesioned rat. *Prog. Brain Res.* **99,** 105–123.
17. Faull R. L. M., Waldvogel H. J., Nicholson L. F. B., Williams M. N. and Dragunow M. (1995) Huntington's disease and neural transplantation: $GABA_A$ receptor changes in the basal ganglia in Huntington's disease in the human brain and in the quinolinic acid lesioned rat model of the disease following fetal neuron transplants. In *Neurotransmitters in the Human Brain*. (eds Tracey D., Stone J. and Paxinos G.), pp. 173–198. Plenum, New York.
18. Ferrante R. J., Beal M. F., Kowall K. W., Richardson E. P. and Martin J. B. (1987) Sparing of acetylcholinesterase-containing striatal neurons in Huntington's disease. *Brain Res.* **411,** 162–166.
19. Ferrante R. J., Kowall N. W., Beal M. F., Richardson E. D., Bird E. D. and Martin J. B. (1985) Selective sparing of a class of striatal neurons in Huntington's disease. *Science* **230,** 561–564.
20. Ferre S., von Euler G., Johansoon B., Fredholm B. B. and Fuxe K. (1991) Stimulation of high affinity adenosine A2 receptors decrease the affinity of dopamine D2 receptors in rat striatal membranes. *Proc. natn. Acad. Sci. U.S.A.* **88,** 7238–7241.
21. Fink J. S., Weaver D. R., Rivkees S. A., Peterfreund R. A., Pollack A. E., Alder E. M. and Reppert S. M. (1992) Molecular cloning of the rat A2 adenosine receptor: selective co-expression with D2 dopamine receptors in rat caudate nucleus and putamen. *Molec. Brain Res.* **14,** 186–195.
22. Gimenez-Amaya J. M. and Graybiel A. M. (1990) Compartmental origins of the striatopallidal projection in the primate. *Neuroscience* **34,** 111–126.
23. Giuffrida A., Parsons L. H., Kerr T. M., Rodríguez De Fonseca F., Navarro M. and Piomelli D. (1999) Dopamine activaton of endogenous cannabinoid signaling in dorsal caudate nucleus and putamen. *Nature Neurosci.* **2,** 358–363.
24. Glass M., Dragunow M. and Faull R. L. M. (1997) Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. *Neuroscience* **77,** 299–318.
25. Glass M., Faull R. L. M. and Dragunow M. (1993) Loss of cannabinoid receptors in the substantia nigra in Huntington's disease. *Neuroscience* **56,** 523–527.
26. Glass M., Faull R. L. M. and Dragunow M. (1996) Localisation of the adenosine uptake site in the human brain: a comparison with the distribution of adenosine A1 receptors. *Brain Res.* **710,** 79–91.
27. Graveland G. A., Williams R. S. and Difiglia M. (1985) Evidence for degenerative and regenerative changes in neostriatal spiny neurons in Huntington's desease. *Science* **227,** 770–773.
28. Graybiel A. M. (1990) Neurotransmitters and neuromodulators in the basal ganglia. *Trends Neurosci.* **13,** 244–254.
29. Hedreen J. C. and Folstein S. E. (1995) Early loss of neostriatal striosome neurons in Huntington's disease. *J. Neuropath. exp. Neurol.* **54,** 105–120.
30. Herkenham M., Lynn A. B., Little M. D., Johnson M. R., Melvin L. S., de Costa B. R. and Rice K. C. (1990) Cannabinoid receptor localization in brain. *Proc. natn. Acad. Sci. U.S.A.* **87,** 1932–1936.
31. Hill M. P., Di Marzo V., Bisogno T., Ambrosino G., Crossman A. R. and Brotchie J. M. (1999) Endocannabinoids and the control of movement and generation of symptoms in Parkinson's disease. *Proceedings of the Symposium on the Cannabinoids*, Burlington, Vermont, International Cannabinoid Research Society, p. 70.
32. Hirsch E. C., Graybiel A. M. and Hersh L. B. (1989) Striosomes and extrastiosomal matrix contain different amounts of immunoreactive choline acetyltransferase in the human caudate nucleus and putamen. *Neurosci. Lett.* **96,** 145–150.
33. James S., Xuereb J. H., Askalan R. and Richardson P. J. (1992) Adenosine receptors in post-mortem human brain. *Br. J. Pharmac.* **165,** 238–244.
34. Le Moine C. and Bloch B. (1995) D1 and D2 dopamine receptor gene expression in the rat caudate nucleus and putamen: sensitive cRNA probes demonstrate prominent segregation of D1 and D2 mRNAs in distinct neuronal populations of the dorsal and ventral caudate nucleus and putamen. *J. comp. Neurol.* **35,** 418–426.
35. Le Moine C., Normand E. and Bloch B. (1991) Phenotypical characterization of the rat striatal neurons expressing the D1 dopamine receptor gene. *Proc. natn. Acad. Sci. U.S.A.* **88,** 4205–4209.

36. Leweke F. M., Gluffrida A., Wurster U., Emrich H. M. and Piomelli D. (1999) Elevated endogenous cannabinoids in schizophrenia. *NeuroReport* **10,** 1665–1669.
37. Mailleux P. and Vanderhaeghen J. J. (1992) Localization of cannabinoid receptor in the human developing and adult basal ganglia. Higher levels in the striatonigral neurons. *Neurosci. Lett.* **148,** 173–176.
38. Martinez-Mir M. I., Probst A. and Palacios J. M. (1991) Adenosine A2 receptors: selective localization in the human basal ganglia and alterations with disease. *Neuroscience* **42,** 697–706.
39. Matsuda L. A., Lolait S. J., Brownstein M. J., Young A. C. and Bonner T. I. (1990) Structure of cannabinoid receptor and functional expression of the cloned cDNA. *Nature* **346,** 561–564.
40. Mayfield R. D., Suzuki F. and Zahniser N. R. (1993) Adenosine A2a receptor modulation of electrically evoked endogenous GABA release from slices of rat globus pallidus. *J. Neurochem.* **60,** 2334–2337.
41. Miller A. S. and Walker J. M. (1995) Effects of a cannabinoid on spontaneous and evoked neuronal-activity in the substantia-nigra pars reticulata. *Eur. J. Pharmac.* **279,** 179–185.
42. Morton A. J., Nicholson L. F. B. and Faull R. L. M. (1993) Compartmental loss of NADPH diaphorase in the neuropil of the human caudate nucleus and putamen in Huntington's disease. *Neuroscience* **53,** 159–168.
43. Myers R. H., Vonsattel J. P., Paskevich P. A., Keily D. K., Stevens T. J., Cupples L. A., Richardson E. P. and Bird E. D. (1991) Decreased neuronal and increased oligodendroglial densities in Huntington's disease caudate nucleus. *J. Neuropath. exp. Neurol.* **50,** 729–742.
44. Nadler V., Biegon A., Beityannai E., Adamchik J. and Shohami E. (1995) Ca-45 accumulation in rat-brain after closed-head injury—attenuation by the novel neuroprotective agent HU-211. *Brain Res.* **685,** 1–11.
45. Oviedo A., Glowa J. and Herkenham M. (1993) Chronic cannabinoid administration alters cannabinoid receptor binding in rat brain: a quantitative autoradiographic study. *Brain Res.* **616,** 293–302.
46. Parent A., Bouchard C. and Smith Y. (1984) The striatopallidal and striatonigral projections: two distinct fibre systems in primate. *Brain Res.* **303,** 385–390.
47. Penney J. B. and Young A. B. (1982) Quantitative autoradiography of neurotransmitter receptors in Huntington's disease. *Neurology* **32,** 1391–1395.
48. Popoli P., Pèzzola A., Reggio R., Caporali M. G. and Scotti de Capolis A. (1994) CGS 21680 antagonises motor hyperactivity in a rat model of Huntington's disease. *Eur. J. Pharmac.* **257,** R5–R6.
49. Reiner A., Albin R. L., Anderson K. D., D'Amato C. J., Penney J. B. and Young A. B. (1988) Differential loss of striatal projection neurons in Huntington's disease. *Proc. natn. Acad. Sci. U.S.A.* **85,** 5733–5737.
50. Reiner A. and Anderson K. D. (1990) The patterns of neurotransmiter and neuropeptide co-occurance among striatal neurons. Conclusions based on recent findings. *Brain Res. Rev.* **15,** 251–265.
51. Reisine T. D., Overstreet D., Gale K., Rossor M., Iversen L. and Yamamura H. I. (1980) Benzodiazepine receptors: the effect of GABA on their characteristics in human brain and their alterations in Huntington's disease. *Brain Res.* **199,** 79–88.
52. Reynolds G. P. and Pearson S. J. (1990) Brain GABA levels in asymptomatic Huntington's disease. *New Engl. J. Med.* **287,** 682.
53. Richfield E. K. and Herkenham M. (1994) Selective vulnerability in Huntington's disease: preferential loss of cannabinoid receptors in lateral globus pallidus. *Ann. Neurol.* **36,** 577–584.
54. Richfield E. K., O'Brien C. F., Eskin T. and Shoulson I. (1991) Heterogenous dopamine receptor changes in early and late Huntington's disease. *Neurosci. Lett.* **132,** 121–126.
55. Sañudo-Peña M. C., Patrick S. L., Patrick R. L. and Walker J. M. (1996) Effects of intranigral cannabinoids on rotational behaviour in rats: interactions with the dopaminergic system. *Neurosci. Lett.* **206,** 21–24.
56. Sañudo-Peña M. C. and Walker J. M. (1997) Role of the subthalamic nucleus in cannabinoid actions in the substantia nigra of the rat. *J. Neurophysiol.* **77,** 1635–1638.
57. Sañudo-Peña M. C. and Walker J. M. (1998) Effects of intrapallidal cannabinoids on rotational behaviour in rats: interactions with dopaminergic systems. *Synapse* **28,** 27–32.
58. Sapp E., Ge P., Aizawa H., Bird E., Penney J., Young A. B., Vonsattel J. P. and Difiglia M. (1995) Evidence for a preferential loss of enkephalin immunoreactivity in the external globus-pallidus in low-grade Huntingtons disease using high-resolution image-analysis. *Neuroscience* **64,** 397–404.
59. Schiffman S. N., Jacobs O. and Vanderhaeghen J. J. (1991) Striatal restricted adenosine A2 receptor (RDC8) is expressed by enkephalin but not be substance P neurons: an *in situ* hybridization histochemistry study. *J. Neurochem.* **57,** 1062–1067.
60. Schiffmann S. N. and Vanderhaeghen J. J. (1993) Adenosine A2 receptors regulate the gene expression of striatopallidal and striatonigral neurons. *J. Neurosci.* **13,** 1080–1087.
61. Spokes E. G. (1980) Neurochemical alterations in Huntington's chorea—a study of postmortem brain tissue. *Brain* **103,** 179–210.
62. Steiner H., Bonner T. I., Zimmer A. M., Kitai S. T. and Zimmer A. (1999) Altered gene expression in striatal projection neurons in CB1 cannabinoid receptor knockout mice. *Proc. natn. Acad. Sci. U.S.A.* **96,** 5786–5790.
63. Storey E. and Beal M. F. (1990) Neurochemical substrates of rigidity and chorea in Huntingtons-disease. *Brain* **116,** 1201–1222.
64. Szabo B., Dörner L., Pfeundtner C., Nörenberg W. and Starke K. (1998) Inhibition of GABAergic inhibitory postsynaptic currents by cannabinoids in rat corpus striatum. *Neuroscience* **85,** 395–403.
65. The Huntington's Disease Collaborative Research Group (1993) A novel gene containing the tri-nucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell* **72**, 971-983.
66. Thibaut F., Hirsch E. C., Raisman R., Javoy-Agid F. and Agid Y. (1990) Microtopography of D1 dopaminergic binding sites in the human substantia nigra: an autoradiographic study. *Neuroscience* **37,** 3873–3898.
67. Vonsattel J-P., Myers R. H., Stevens T. J., Ferrante R. J., Bird E. D. and Richardson E. P. (1985) Neuropathological classification of Huntington's disease. *J. Neuropath. exp. Neurol.* **44,** 559–577.
68. Yoles E., Belkin M. and Schwartz M. (1993) HU-211, a nonpsychotropic cannabinoid, produces short- and long-term neuroprotection after optic nerve axotomy. *J. Neurotrauma* **13,** 49–57.

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