Striosomes and mood dysfunction in Huntington’s disease

Lynette J. Tippett,1 Henry J. Waldvogel,2 Sally J. Thomas,1 Virginia M. Hogg,1 Willeke van Roon-Mom,2 Beth J. Synek,3 Ann M. Graybiel4 and Richard L. M. Faull2

1Department of Psychology, 2Department of Anatomy with Radiology, The University of Auckland, 3Department of Forensic Pathology, Auckland City Hospital, Auckland, New Zealand and 4Department of Brain and Cognitive Sciences and the McGovern Institute for Brain Research, Massachusetts Institute of Technology, Cambridge, MA, USA

Correspondence to: Dr Lynette J. Tippett, Department of Psychology, The University of Auckland, Private Bag 92019, Auckland, New Zealand
E-mail: l.tippett@auckland.ac.nz

Variable phenotype is common in neurological disorders with single-gene inheritance patterns. In Huntington’s disease, mood and cognitive symptoms are variably co-expressed with motor symptoms. There is also variable degeneration of neurons in the two major neurochemical compartments of the striatum, the striosomes and the extrastriosomal matrix. To determine whether the phenotypic variability in Huntington’s disease is related to this compartmental organization, we carried out a double-blind study in which we used GABA_A receptor immunohistochemistry to analyse the status of striosomes and matrix in the brains of 35 Huntington’s disease cases and 13 control cases, and collected detailed data on the clinical symptomatology expressed by the patients from family members and records. We report here a significant association between pronounced mood dysfunction in Huntington’s disease patients and differential loss of the GABA_A receptor marker in striosomes of the striatum. This association held for both clinical onset and end-stage assessments of symptoms. The cases with accentuated striosome abnormality further exhibited later onset age, lower disease grade and lower CAG repeat length in the HD gene. We found no independent association, however, between CAG repeat length or age of onset and mood dysfunction. We suggest that variation in clinical symptomatology in Huntington’s disease is associated with variation in the relative abnormality of GABA_A receptor expression in the striosome and matrix compartments of the striatum, and that striosome-related circuits may modulate mood functioning.

Keywords: striosomes, striatum; neurological disorder; Huntington’s disease; mood symptoms

Abbreviations: NeuN = neuronal N; PBS = phosphate-buffered saline


Introduction

A key interface between neuroscience and neurology has emerged with the identification of gene mutations inducing neurological disorders with characteristic behavioural phenotypes. Among such disorders, Huntington’s disease is prototypic. A CAG expansion in a single gene, IT15, results in an autosomal-dominant, progressive neurodegenerative disorder producing massive cell death in the forebrain and a severe choreoathetotic disorder with behavioural symptoms. Despite the single-gene aetiology of Huntington’s disease, however, there is remarkable variability in the types of behavioural symptoms present in different Huntington’s disease patients both at clinical onset and thereafter (Andrew et al., 1993; Claes et al., 1995). Some patients mainly exhibit motor dysfunction at clinical onset, and few, if any, changes in mood for extended periods of time. Other patients experience severe changes in mood and/or cognitive function at clinical onset, but have fewer abnormal movements. Still others experience concomitant motor, mood and cognitive symptoms, now considered the classic triad of Huntington’s disease symptomatology (Brandt and Butters, 1986; Folstein, 1989; Myers et al., 1991a; Claes et al., 1995; Zappacosta et al., 1996; Thompson et al., 2002). A compelling demonstration of this clinical variability was documented for monozygotic twins, who inherited identical HD genes but exhibited marked differences in their behavioural symptoms (Georgiou et al., 1999). The onset of clinical symptoms in individual
Striosomes and mood in Huntington’s disease

patients is roughly correlated with the number of CAG repeats (Wexler et al., 2004), but no consistent relationship has been found across studies between CAG repeat length and symptom subtype (MacMillan et al., 1993; Telenius et al., 1994; Claes et al., 1995; Zappacosta et al., 1996). Thus the source of variability in symptom subtypes is not clear.

Neurodegeneration in Huntington’s disease occurs most prominently in the basal ganglia and neocortex. In the basal ganglia, the tissue mass of the striatum (caudate nucleus and putamen) becomes cavitated, leaving only ventral striatal regions relatively intact. Key patterns of cell death in the striatum are consistent across Huntington’s disease cases. First, it is mainly the GABAergic projection neurons of the striatum that undergo cell death, so that loss of the output tracts of the striatum predominates. Neurons that project to the external pallidum tend to degenerate before those projecting to the internal pallidum and substantia nigra (Reiner et al., 1988; Albin et al., 1992; Faull et al., 1993; Glass et al., 1993; Glass et al., 2000; Deng et al., 2004). Further, the progression of neuronal degeneration follows anatomical gradients across the cardinal dimensions of the striatum (Ferrante et al., 1987; Vonsattel et al., 1997). It remains a matter of controversy, however, whether there is selective degeneration of neurons in either of the two major neurochemical compartments of the striatum: the striosomes and matrix (Graybiel and Ragsdale, 1978; Ferrante et al., 1987; Feigenbaum and Graybiel, 1988; Seto-Ohshima et al., 1988; Morton et al., 1993; Hedreen and Folstein, 1995). Some studies report preferential loss of neurons and neurochemical markers in the matrix compartment early in the progression of Huntington’s disease (Olsen et al., 1986; Ferrante et al., 1987; Seto-Ohshima et al., 1988; Faull et al., 1993), but others report that neurons and neurochemical markers are lost in striosomes, with clear sparing of the matrix, at least early on (Morton et al., 1993; Hedreen and Folstein, 1995; Augood et al., 1996).

We reasoned that if these divergent findings reflect true heterogeneity in the patterns of neurodegeneration in the brains of different Huntington’s disease patients, then the heterogeneity might provide a key to analysis of the clinical variability in symptomatology. Studies of the non-human primate have shown differential patterns of connectivity for the striosome and matrix compartments in the striatum (Flaherty and Graybiel, 1994; Graybiel et al., 1994; Ebben and Graybiel, 1995). Striosomes are interconnected with regions linked to limbic circuitry, and the matrix is interconnected with sensorimotor and associative cortical circuitry. If these patterns of connectivity have functional significance, differential dysfunction of striosome-based circuits might thus contribute to the differential appearance of mood disorders in Huntington’s disease, and differential dysfunction of matrix-based pathways to motor symptoms. We tested for such relationships in a double-blind study by comparing the compartmental patterns of striatal abnormality found post-mortem with the patterns of mood and motor symptoms exhibited by the patients at clinical onset and clinical end stage as determined by retrospective analyses. Our strategy for the anatomical analysis was to combine immunostaining for the β2,3 subunits of the GABA<sub>A</sub> receptor, which specifically labels the class of striatal GABAergic neurons that are affected in Huntington’s disease (Waldvogel et al., 1999) and labels both striosomes and matrix, with immunostaining selective for each compartment (Holt et al., 1997; Graybiel and Penney, 1999; Gunterkunst et al., 2002). For the clinical analysis, we combined data from interviews and questionnaires obtained from family members. Our findings demonstrate that patients with predominant GABA<sub>A</sub> receptor loss in striosomes exhibited differentially pronounced mood dysfunction at both early and late stages of the disease and that these patients on average exhibited a milder disease course. We suggest that different patterns of dysfunction in the striosome and matrix compartments of the striatum and their corresponding neural circuits could contribute significantly to the variability in behavioural symptomatology experienced in Huntington’s disease.

Material and methods

All protocols used in this study were approved by the University of Auckland Human Participants Ethics Committee and the Auckland Ethics Committees, and informed consent was obtained from all families.

Neuroanatomical studies

Brain tissue from 35 Huntington’s disease cases (Table 1) and 13 control cases (Table 2) was obtained from the Neurological Foundation of New Zealand Human Brain Bank in the Department of Anatomy with Radiology, University of Auckland. The disease cases included 20 males and 15 females aged 39–82 years (average 60.3 years) with a post-mortem interval between 2.5 and 41 h (average 14.9 h). The control cases included 10 males and 3 females, aged 46–82 years (average 64.1 years) with a post-mortem interval between 5 and 21 h (average 14.3 h).

Brains were perfused through the basilar and carotid arteries, first with phosphate-buffered saline (PBS) and 1% sodium nitrite, followed by fixation with 15% formalin in 0.1 M phosphate buffer, pH 7.4, for 1 h. Following perfusion, blocks containing the basal ganglia were dissected out and kept in the same fixative for 24 h. The blocks were then cryo-protected in 20% sucrose in 0.1 M phosphate buffer with 0.1% Na-azide for a further 2–3 days and then were stored in a –80°C freezer until further processing. Additional blocks were taken for pathological examination. The neuropathological grading of the disease cases was undertaken according to the standard Vonsattel grading criteria (grades 0–4) (Vonsattel et al., 1985; Myers et al., 1991b; Vonsattel and DiFiglia, 1998) by a neuropathologist (B.L.S.) with extensive familiarity with Huntington’s disease neuropathology. Similarly blocked control tissue was obtained from cases with no history of neurological disease that, on pathological examination, showed no abnormalities. For each disease case, the number of CAG repeats in both alleles of the IT15 gene was determined as described in Whitefield et al. (Whitefield et al., 1996) by polymerase chain reaction (PCR) amplification of DNA isolated from blood samples or cerebellar brain tissue from the same brains.
For immunohistochemistry, blocks containing the basal ganglia at the level of the caudate–putamen complex and the globus pallidus were cut at 70 μm on a freezing microtome and were processed free-floating in tissue culture wells. Adjacent series of sections were washed in PBS and 0.2% Triton-X (PBS-Triton), incubated for 20 min in 50% methanol and 1% H₂O₂, washed (3·15 min) in PBS-Triton and then incubated in primary antibody for 2–3 days on a shaker at 4°C. The primary antibodies used were as follows: mouse monoclonal antibody bd-17, which recognizes the β₂,β₃ subunits of the GABAA receptor (kindly donated by J.-M. Fritschy and H. Möhler, Institute of Pharmacology and Toxicology, University of Zurich, Switzerland, diluted 1:20 000) (Haring et al., 1985; Schoch et al., 1985; Ewert et al., 1990), mouse monoclonal antibody against enkephalin (Seralab, Poole, UK, diluted 1:10 000) to mark striosomes, rabbit polyclonal against the calcium binding protein calbindin (kindly donated by Dr Piers Emson, Babraham Institute, Cambridge, UK, diluted 1:2000) or mouse monoclonal antibody against the calcium binding protein calbindin (SWANT, Bellinzona, Switzerland, diluted 1:500) to mark the extrastriosomal matrix compartment of the striatum (Graybiel, 1990; Holt et al., 1997; Waldvogel et al., 1999) and monoclonal antibody neuronal N (NeuN) (Chemicon, 1:1000) to mark neurons. Sections were then washed (3·15 min PBS-Triton) and incubated overnight in the appropriate species-specific biotinylated secondary antibody, sheep anti-rabbit (Chemicon, diluted 1:500) or goat anti-mouse (Sigma, diluted 1:500). Following washing (3·15 min PBS-Triton), the sections were incubated for 4 h at room temperature in either streptavidin–conjugated HRP

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Gender</th>
<th>Age at onset</th>
<th>Age at death</th>
<th>CAG repeat length IT15 gene</th>
<th>P/M interval (hours)</th>
<th>Grade</th>
<th>Compartment mainly affected</th>
<th>Cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC46</td>
<td>M</td>
<td>52</td>
<td>59</td>
<td>43/16</td>
<td>2.5</td>
<td>0</td>
<td>S</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>HC48</td>
<td>M</td>
<td>41</td>
<td>62</td>
<td>47/17</td>
<td>20</td>
<td>3</td>
<td>M</td>
<td>Septicaemia</td>
</tr>
<tr>
<td>HC50</td>
<td>F</td>
<td>43</td>
<td>66</td>
<td>42/20</td>
<td>9</td>
<td>1</td>
<td>SM</td>
<td>Subdural haematoma</td>
</tr>
<tr>
<td>HC51</td>
<td>M</td>
<td>40</td>
<td>58</td>
<td>43/16</td>
<td>4.5</td>
<td>1</td>
<td>S</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>HC52</td>
<td>F</td>
<td>31</td>
<td>61</td>
<td>46/19</td>
<td>23</td>
<td>2</td>
<td>M</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>HC53</td>
<td>M</td>
<td>41</td>
<td>56</td>
<td>43/17</td>
<td>14</td>
<td>1</td>
<td>M</td>
<td>Bowel obstruction</td>
</tr>
<tr>
<td>HC54</td>
<td>M</td>
<td>31</td>
<td>56</td>
<td>NA</td>
<td>14</td>
<td>2</td>
<td>SM</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>HC55</td>
<td>M</td>
<td>76</td>
<td>87</td>
<td>42/14</td>
<td>20</td>
<td>1</td>
<td>S</td>
<td>Peritonitis</td>
</tr>
<tr>
<td>HC57</td>
<td>M</td>
<td>39</td>
<td>58</td>
<td>47/18</td>
<td>29</td>
<td>2</td>
<td>S</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>HC58</td>
<td>M</td>
<td>39</td>
<td>64</td>
<td>44/18</td>
<td>19</td>
<td>3</td>
<td>S</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>HC60</td>
<td>M</td>
<td>41</td>
<td>64</td>
<td>43/18</td>
<td>23</td>
<td>1</td>
<td>S</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>HC62</td>
<td>F</td>
<td>58</td>
<td>78</td>
<td>43/18</td>
<td>6</td>
<td>2</td>
<td>S</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>HC64</td>
<td>M</td>
<td>18</td>
<td>47</td>
<td>51/19</td>
<td>41</td>
<td>3</td>
<td>SM</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>HC65</td>
<td>F</td>
<td>41</td>
<td>82</td>
<td>41/18</td>
<td>19</td>
<td>2</td>
<td>SM</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>HC66</td>
<td>M</td>
<td>52</td>
<td>62</td>
<td>41/27</td>
<td>19</td>
<td>0</td>
<td>S</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>HC70</td>
<td>M</td>
<td>24</td>
<td>39</td>
<td>52/18</td>
<td>15</td>
<td>3</td>
<td>M</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>HC72</td>
<td>F</td>
<td>36</td>
<td>63</td>
<td>42/17</td>
<td>24</td>
<td>2</td>
<td>M</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>HC74</td>
<td>M</td>
<td>17</td>
<td>42</td>
<td>42/17</td>
<td>12</td>
<td>1</td>
<td>S</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>HC75</td>
<td>M</td>
<td>21</td>
<td>41</td>
<td>52/18</td>
<td>5.5</td>
<td>2</td>
<td>M</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>HC76</td>
<td>M</td>
<td>53</td>
<td>71</td>
<td>42/19</td>
<td>16</td>
<td>2</td>
<td>SM</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>HC78</td>
<td>M</td>
<td>25</td>
<td>48</td>
<td>45/20</td>
<td>18</td>
<td>3</td>
<td>M</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>HC79</td>
<td>F</td>
<td>49</td>
<td>56</td>
<td>42/17</td>
<td>4</td>
<td>1</td>
<td>S</td>
<td>Respiratory failure</td>
</tr>
<tr>
<td>HC80</td>
<td>F</td>
<td>35</td>
<td>45</td>
<td>43/23</td>
<td>15</td>
<td>2</td>
<td>SM</td>
<td>Asphyxia</td>
</tr>
<tr>
<td>HC81</td>
<td>F</td>
<td>40</td>
<td>70</td>
<td>41/19</td>
<td>8</td>
<td>1</td>
<td>S</td>
<td>Heart failure</td>
</tr>
<tr>
<td>HC82</td>
<td>M</td>
<td>50</td>
<td>74</td>
<td>42/15</td>
<td>16</td>
<td>2</td>
<td>S</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>HC83</td>
<td>M</td>
<td>40</td>
<td>80</td>
<td>40/20</td>
<td>9</td>
<td>1</td>
<td>S</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>HC84</td>
<td>F</td>
<td>15</td>
<td>43</td>
<td>49/21</td>
<td>3.5</td>
<td>3</td>
<td>M</td>
<td>Acute renal failure</td>
</tr>
<tr>
<td>HC85</td>
<td>F</td>
<td>44</td>
<td>61</td>
<td>44/24</td>
<td>19</td>
<td>3</td>
<td>M</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>HC87</td>
<td>M</td>
<td>27</td>
<td>45</td>
<td>50/21</td>
<td>18</td>
<td>4</td>
<td>M</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>HC88</td>
<td>F</td>
<td>44</td>
<td>57</td>
<td>44/17</td>
<td>19</td>
<td>2</td>
<td>SM</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>HC89</td>
<td>F</td>
<td>64</td>
<td>71</td>
<td>44/17</td>
<td>12</td>
<td>2</td>
<td>M</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>HC91</td>
<td>F</td>
<td>35</td>
<td>63</td>
<td>44/23</td>
<td>5.5</td>
<td>3</td>
<td>SM</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>HC92</td>
<td>M</td>
<td>40</td>
<td>72</td>
<td>41/17</td>
<td>5</td>
<td>1</td>
<td>S</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>HC93</td>
<td>F</td>
<td>32</td>
<td>56</td>
<td>43/20</td>
<td>17</td>
<td>3</td>
<td>M</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>HC94</td>
<td>M</td>
<td>38</td>
<td>54</td>
<td>47/17</td>
<td>16</td>
<td>2</td>
<td>SM</td>
<td>Pneumonia</td>
</tr>
</tbody>
</table>

Table 2 Normal control cases

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Gender</th>
<th>Age at death</th>
<th>P/M delay (hours)</th>
<th>Cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>H103</td>
<td>Male</td>
<td>70</td>
<td>5</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>H111</td>
<td>Male</td>
<td>46</td>
<td>10</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>H115</td>
<td>Male</td>
<td>61</td>
<td>12</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>H135</td>
<td>Male</td>
<td>62</td>
<td>21</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>H140</td>
<td>Male</td>
<td>51</td>
<td>18</td>
<td>Cardiomyopathy</td>
</tr>
<tr>
<td>H155</td>
<td>Male</td>
<td>61</td>
<td>7</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>H164</td>
<td>Male</td>
<td>82</td>
<td>21</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>H229</td>
<td>Female</td>
<td>76</td>
<td>21</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>H265</td>
<td>Male</td>
<td>71</td>
<td>15</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>H310</td>
<td>Male</td>
<td>72</td>
<td>10.5</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>H334</td>
<td>Female</td>
<td>60</td>
<td>12</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>H466</td>
<td>Female</td>
<td>53</td>
<td>17</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>H530</td>
<td>Male</td>
<td>68</td>
<td>16.5</td>
<td>Myocardial infarction</td>
</tr>
</tbody>
</table>
complex (Chemicon, 1 : 1000) for the rabbit polyclonal antibody, or ExtrAvidin™ (Sigma, 1 : 1000) for the mouse antibody. The sections were then exposed to 0.05% 3,3-diaminobenzidine tetrahydrochloride (DAB, Sigma) and 0.01% \( \text{H}_2\text{O}_2 \) in 0.1 M phosphate buffer, pH 7.4, for 15–20 min to produce a brown reaction product. A nickel-intensification procedure was also used, in which 0.4% nickel ammonium sulphate was added to the DAB solution to produce a blue–black reaction product (Adams, 1981). The sections were washed in PBS, mounted on chrome-alum-coated slides, rinsed in distilled water, dehydrated through a graded alcohol series to xylene and cover-slipped with Hystomount (Hughes and Hughes, UK). Some sections were processed as controls to determine non-specific staining by following the same immunohistochemical procedures except for omission of the primary antibody/antiserum. To minimize variability in staining, the processing and storage of the brains and treatments of the sections were carried out as nearly identically as possible from case to case, and normal control tissue sections were included in the processing of sections from disease cases as positive controls. Anatomical analysis was carried out by investigators blind to the clinical data. The assignment of cases to the anatomical groups described was undertaken separately by two or three independent investigators with expertise in the immunohistochemical compartmentalization of the human striatum.

Methods to assess clinical symptomatology

Clinical data were collected retrospectively from family members of the 35 individuals who had died with Huntington’s disease and whose families had requested donation of their brain tissue to the New Zealand Neurological Foundation Human Brain Bank. The clinical data were collected via a semi-structured interview and a questionnaire, both administered by researchers blind to the neuroanatomical analyses of the brains. Interviews were conducted with one (n = 19) or more than one (n = 16) close family member. Retrospective clinical data were available for all but two of the 35 patients at symptom onset and for all patients at end stage. Clinical onset was defined as the first significant enduring change in functioning in motor, mood or cognitive domains that occurred during the life history obtained for each individual. Determination of onset required the presence of specific exemplars of behaviours consistent with Huntington’s disease symptomatology when these marked the beginning of a persistent and continuing pattern of change for the individual. Although current clinical diagnosis of this disease in living individuals typically requires the presence of unequivocal motor abnormalities (Huntington Study Group, 1996), it is widely accepted that Huntington’s disease may alternatively present with mood or with psychiatric symptoms, that cognitive decline may precede the development of unequivocal motor signs and that the earliest motor symptoms may not be chorea (Paulsen et al., 2001; Squitieri et al., 2001). In our study, documentation of the functional, behavioural and motor performance history across the total lifespan of each Huntington’s disease case made it possible to determine whether an occurrence of a major functional change (for example, presence of marked mood or psychiatric symptoms) was a temporally restricted single episode or set of episodes, or whether it represented the onset of a new, persistent pattern of functional change for that individual. This enabled us to consider symptoms from all three functional domains when estimating clinical onset.

Semi-structured interview

The interview format was designed to facilitate the collection of accurate information about the age of clinical onset and the patterns of clinical change related to the disease for each Huntington’s disease case. The interviews were structured so that the initial questions were open and broad and subsequent questions became more specific. This design enabled interviewees to tell their version of events before being exposed to the prompts and possible constraints of specific questions. The interview design also enabled the interview to be fluid, progressing naturally from the interviewee’s story to more specific details about their relative’s experiences.

Clinical Huntington’s Disease Questionnaire

A clinical questionnaire was developed specifically for this study in order to provide a comprehensive representation of all reported changes observed in Huntington’s disease, and to provide a way to estimate the severity of impairment in the primary domains of clinical change in the disease. The questionnaire consisted of 49 specific questions about the presence or absence of difficulties experienced by the patients during the clinical course of the disease, posed in language readily understandable to the lay person (Supplementary Table 1 in Brain online).

For each item, interviewees indicated whether or not the behaviour change or symptom described was seen in the family member with the illness at two stages of the disease (at clinical onset, and in the period near death). A ‘don’t know’ option was also present. If a symptom was indicated as being present in either of the two stages, the interviewee was required to score its severity on a 5-point scale (extremely mild, mild, moderate, severe or extremely severe). Qualitative descriptions of each point on the severity scale were provided as a guide for differences between the five possible rating options (Table 3).

Items in the questionnaire were classified as reflecting motor, mood or cognitive impairments on the basis of multiple criteria. First, four experienced neuropsychologists independently classified each item as reflecting impairments in one of the three clinical domains, in none of the three domains or in more than one domain. Items for which there was 100% agreement among all four raters, and that were classified as uniquely reflecting motor or mood impairment, were retained for estimating severity of impairment within these domains. This left 17 items in the motor domain and 10 in the mood domain. Secondly, Spearman’s correlation coefficients were calculated between responses on all items at both time points (clinical onset and end stage) to determine

<table>
<thead>
<tr>
<th>Severity</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extremely mild</td>
<td>Not present often/only noticeable occasionally</td>
</tr>
<tr>
<td>Mild</td>
<td>Present a lot of the time/not causing incapacity</td>
</tr>
<tr>
<td>Moderate</td>
<td>Present most/all of the time to some extent. Interfering with some activities</td>
</tr>
<tr>
<td>Severe</td>
<td>Present all the time. Prevents a lot of activities/needs treatment</td>
</tr>
<tr>
<td>Extremely severe</td>
<td>Present all of the time to a large extent. Severe impact on activities/functioning</td>
</tr>
</tbody>
</table>
Table 4: Items from Clinical Huntington’s Disease Questionnaire contributing to motor and mood indices

<table>
<thead>
<tr>
<th>Motor items</th>
<th>Mood items</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Sudden, quick, jerky, unintended movements of almost any part of body</td>
<td>1. Depressed. (Possible indicators include low mood; feelings of guilt, hopelessness, or worthlessness; loss of interest and energy; loss of appetite; insomnia; decreased participation in social activities; and decreased sexual interest.)</td>
</tr>
<tr>
<td>2. Slower, writhing movements. (Early in the course of the disease, these movements are often mistaken for nervousness or mannerisms. They may first be noticeable when the person starts to do something and may involve flexing and extending the fingers and facial ticks.)</td>
<td>2. Suicidal thoughts or actions</td>
</tr>
<tr>
<td>3. Restless. (The persons may appear nervous, fidget, be unable to sit quietly, or they may gesture (make expressive hand and arm movements) more than they used to.)</td>
<td>3. Irritable (uncharacteristically irritable in response to issue or situation that does not warrant such an outburst.)</td>
</tr>
<tr>
<td>4. Holding finger, hand, arm, leg or upper body part in an awkward position.</td>
<td>4. Anxious (e.g. worries excessively, often about ‘minor issues’, or, social interactions)</td>
</tr>
<tr>
<td>5. Tremor (regular rhythmic action) [showing a fine tremor, especially noticeable in arms (when holding arms straight out in front of body)]</td>
<td>5. Compulsive/obsessional behaviour [e.g. accumulating things (such as food, or worthless items) or excessive repetitive activity, such as washing of hands or body]</td>
</tr>
<tr>
<td>6. Difficulty continuing a physical activity over a period of time. (The person has difficulty or is unable to keep doing the same physical activity over a period of time, for example, being unable to maintain their gaze on a person or object, or dropping a cup of coffee because they are unable to keep holding it.)</td>
<td>6. Delusional (implausible, bizarre beliefs held by the person)</td>
</tr>
<tr>
<td>7. Slow, or different, eye movements. (Eyes seem to move slowly and are different from normal.)</td>
<td>7. Greatly increased activity level/extremely happy mood</td>
</tr>
<tr>
<td>8. Less able to use hands for tasks involving fine movements. (In daily life the person may be clumsy and have difficulty using tools, tying shoelaces, buttoning clothes, cutting food and putting dishes in and out of the oven.)</td>
<td>8. Wakes up frequently during sleep time</td>
</tr>
<tr>
<td>9. Speech is slow, slurred or unclear.</td>
<td></td>
</tr>
<tr>
<td>10. The rhythm of speech is not smooth (there may be bursts of words and pauses half way through sentences).</td>
<td></td>
</tr>
<tr>
<td>11. Difficulty swallowing</td>
<td></td>
</tr>
<tr>
<td>12. Spilling food and noisy eating and drinking</td>
<td></td>
</tr>
<tr>
<td>13. Choking</td>
<td></td>
</tr>
<tr>
<td>14. Jerky, uneven, slow walk with short steps</td>
<td></td>
</tr>
<tr>
<td>15. Sudden knee bends when walking</td>
<td></td>
</tr>
<tr>
<td>16. Appears to ‘lurch’ when walking (might look like a drunken stagger)</td>
<td></td>
</tr>
<tr>
<td>17. Restless while sleeping</td>
<td></td>
</tr>
</tbody>
</table>

Collinearity. Pairs of items within a domain with correlations of 0.8 or higher were reduced to a single item. Two pairs of items within the mood domain met this criterion, and thus were reduced accordingly. The final list of items measuring domain-specific symptoms contained 25 items: 17 in the motor domain (6 reflecting involuntary movements and 11 reflecting voluntary movements) and 8 in the mood domain (Table 4 and Supplementary Table 1 in Brain online). The items retained in the mood domain did not tap apathy or related behaviours, as these were judged to be ambiguous with regard to their underlying impairment (i.e. they could reflect mood disturbance or cognitive impairment). Thus the mood items included predominantly reflected anxiety, depression, repetitive behaviour and irritability. For each Huntington’s disease case, severity ratings for mood items and motor items at each time point (clinical onset and end stage) were converted into numerical ratings (0 for ‘not present’ to 5 for ‘extremely severe’), which were then summed and converted to an index out of 100. The higher the score, the greater the impairment.

To estimate the validity of the clinical questionnaire, it was administered to family members of individuals with clinical signs of Huntington’s disease for whom clinical measures had previously been gathered by a neurologist who administered the Quantified Neurological Examination (QNE) (Folstein et al., 1983) and a clinical psychologist who administered formal measures of mood using the Hospital Anxiety and Depression Scale (Zigmond and Snaith, 1983) and the Irritability, Depression and Anxiety Scale (Snaith et al., 1978). The interval between the times for which symptoms were assessed and the administration of the questionnaire in this control study ranged from 12 to 64 months (m = 28.7 months, SE = 6.74). This interval was comparable with the interval between death of the Huntington’s disease patients and the time of the clinical assessments in the main study reported here (m = 28.7 months, SE = 3.02, range = 2–63 months). Spearman’s correlations demonstrated significant correlations between the Clinical Huntington’s Disease Motor Index and the total QNE score (r = 0.75, P = 0.04, one-tailed), and the Clinical Huntington’s Disease Mood Index and Hospital depression score (r = 0.71, P = 0.04, one-tailed), providing preliminary evidence of good validity for the end-stage motor and mood indices in the questionnaire.

Procedure for acquisition of clinical data

Potential interviewees were identified by the director of the Neurological Foundation of New Zealand Human Brain Bank from records of donors to the Brain Bank. When approached about the research, 100% of these individuals agreed to participate in the study. Interviews were conducted at homes beginning with the semi-structured interview, followed by the Clinical Huntington’s Disease Questionnaire, which was administered with the assistance of the researcher who clarified the content of individual items and helped explain the severity scale. Any inconsistencies between information given in the semi-structured interview and responses on the questionnaire were investigated at the end of the interview. The researcher conducting this part of the study was blind to the anatomical data obtained for Huntington’s disease cases.

Statistical methods

Comparisons among the anatomically classified groups on the indices of mood and motor dysfunction were performed with two-tailed Mann–Whitney U-tests. Pearson’s correlations were conducted between CAG repeat length, age of clinical onset, age of death and Vonsattel grade of pathology. A multivariate analysis of variance (MANOVA) was then used to compare striosomal loss, matrix-loss and mixed-loss groups on these variables. Partial correlations controlling for compartment loss were computed to test for associations between mood disturbance and CAG repeat length and mood disturbance and age. Finally, a multiple regression
Striosomes and mood in Huntington’s disease

analysis was performed to determine predictors of mood dysfunction. All analyses were conducted using the statistical package SPSS, Version 12.0.1.

Results

Patterns of compartmental loss in the Huntington’s disease brain

We obtained behavioural assessments and post-mortem anatomical analyses for 35 Huntington’s disease cases. In addition, we obtained post-mortem anatomical analyses for 13 control cases roughly matched for age and post-mortem delay, in which no neuropathological changes were found. The brains of the cases represented the full range of neuropathological severity in Huntington’s disease (Vonsattel stages 0–4) and had disease allele CAG repeat lengths ranging from 40 to 52 (Table 1).

The pattern of GABA_A receptor immunostaining demonstrated highly non-uniform patterns of GABA_A receptor loss in the dorsal striatum across the Huntington’s disease cases (Fig. 1). As shown in Fig. 1A, the bd-17 monoclonal antibody labels both striosomes and matrix in the striatum of neurologically normal individuals, but small regions of heightened or encapsulated immunostaining appear, and these correspond to striosomes identified in adjoining sections by enhanced enkephalin immunostaining and diminished calbindin immunostaining relative to the surrounding tissue (Figs 2A–C and 6A–C; see also Waldvogel et al., 1999). The surrounding background regions of moderate to dense GABA_A receptor immunostaining correspond to the extrastriosomal matrix identifiable by enhanced calbindin immunostaining (Figs 2B and 6B).

This widespread immunostaining of both compartments in the GABA_A sections was critical to our analysis because it allowed us to detect depletions of staining that were focal and presumptively corresponded to striosomes, and also regions of focal retention of the GABA_A immunostaining in a surround of reduced immunostaining. By comparing the GABA_A receptor immunostaining with both striosome-enriched (enkephalin) and matrix-enriched (calbindin) immunostaining, we were able to identify regions of differential retention or loss of the GABA_A receptor staining as corresponding either to striosomes or to matrix. The cases designated as having predominant striosome loss were distinguished by conspicuous pockets of low GABA_A receptor immunostaining that were surrounded by regions of moderate to high densities of the GABA_A receptor staining (Figs 1B, 3A and 5A and E). The pockets of GABA_A receptor loss corresponded to striosomes identified as focal zones of differentially low calbindin immunostaining (Figs 3A and B, 5A, B, E and F); the extensive loss of GABA_A receptor staining in the striosomes was also matched by a corresponding loss of enkephalin staining in adjacent sections, which normally identifies intact striosomes (Fig. 3A and C). These correspondences were readily appreciated at high magnification (Figs 4–7). The levels of calbindin and GABA_A receptor immunostaining in the matrix of these striosome-loss cases suggested that the striosome depletion of GABA_A receptors was accompanied by at least some loss in the matrix. Especially dorsally, there was generally some weakening of immunostaining in the matrix, together with severe depletion in striosomes.

The matrix-loss cases exhibited a distinctive pattern in which clearly delineated foci of strong GABA_A receptor

Fig. 1  Comparison of the patterns of GABA_A receptor β2,3 subunit immunostaining in the striatum of a normal case (A) and three Huntington’s disease cases with predominant receptor loss in the striosome compartment (B), in the matrix compartment (C) or in both striatal compartments (D). Each photomicrograph illustrates a cross-section through the caudate nucleus and putamen from the cases indicated. (A) Case H115, showing that GABA_A receptor immunostaining in the normal brain is distributed throughout the caudate nucleus and putamen with slightly higher densities of immunostaining, or encapsulated zones of staining, in the striosomes (examples at asterisks, also in B–D) and moderately high densities of staining in the matrix (examples indicated by m).

(B) Case HC79 (grade 1), a striosome-loss case with prominent loss of GABA_A receptor immunostaining mainly in the striosomal compartment (see also Figs 3A–C and 6D–F). (C) Case HC78 (grade 3), a matrix-loss case with extensive depletion of GABA_A receptor immunostaining in the matrix compartment (see also Fig. 4A–D). (D) Case HCS6 (grade 3), exhibiting reduced GABA_A receptor immunostaining in both striosome and matrix compartments. CN = caudate nucleus; IC = internal capsule; P = putamen. Scale bars = 5 mm.
Fig. 2 Low power photomicrographs illustrating GABA<sub>A</sub> receptor immunostaining and immunostaining for neurochemical markers of striosome and matrix compartments in nearby sections from the striatum of normal case H265 (A–C) and, in D–F, Huntington’s disease case HC65 (grade 2) showing mixed compartmental loss of GABA<sub>A</sub> receptor immunostaining. The distribution of GABA<sub>A</sub> receptor is shown in A and D. Calbindin immunostaining, a marker for the matrix compartment, is shown in B and E. Striosomes appear as pale zones. Enkephalin immunostaining, marking striosomes, is shown in C and F. Striosomes appear as dark zones. In the normal brain moderately dense GABA<sub>A</sub> receptor immunostaining is distributed through the caudate nucleus and putamen (A). Small zones of slightly dense immunostaining are present, which closely match striosomes (example at asterisk) visible in closely spaced sections stained for calbindin (B) and enkephalin (C). In the Huntington’s disease brain (D–F), there is a generalized loss of these neurochemical markers in the dorsal regions of the striatum that affects both striosomes and matrix (hence the classification as a mixed-loss case). Asterisks indicate examples of striosomes. Outlined regions are illustrated at higher magnification in Fig. 6A–C and J–L. CN = caudate nucleus; IC = internal capsule; P = putamen. Scale bars = 5 mm.

Fig. 3 Low power photomicrographs illustrating the compartmental distribution of neurochemical markers in the striatum in closely spaced sections from Huntington’s disease case HC79 (grade 1) with predominant striosomal loss of GABA<sub>A</sub> receptor immunostaining and case HC87 (grade 4) with predominant loss of GABA<sub>A</sub> receptor immunostaining in the matrix. (A and D) Distribution of immunostaining for the β<sub>2,3</sub> subunit of the GABA<sub>A</sub> receptor. (B and E) Distribution of calbindin immunostaining. (C and F) Distribution of enkephalin immunostaining. In case HC79 (A–C) there is differential loss of GABA<sub>A</sub> immunostaining in the striosomal compartment of the dorsal striatum, whereas in HC87 (D–F) there is massive loss of neurochemical markers in the matrix compartment. Both cases illustrate that although there is differential loss of GABA<sub>A</sub> receptor immunostaining in either striosomes or matrix, depletion of immunostaining also has occurred in the complementary compartment. Asterisks indicate examples of striosomes. Outlined regions are shown at higher magnification in Fig. 6D–I. Scale bars = 5 mm.
staining appeared against a background of low receptor staining (Figs 1C, 3D and 4A and E). These zones of high receptor staining corresponded to the preserved striosomes visible with enkephalin immunostaining in adjacent sections (Figs 3D and F, 4 and 6G and I). The extensive loss of GABA<sub>A</sub> receptor immunostaining in the background (Figs 1C, 3D, 4A and E and 6G) was mirrored by a corresponding loss of calbindin staining in adjacent sections, which normally distinguishes the intact matrix (Figs 3D and E and 6G and H). The pattern of compartmental loss was again relative. Vivid GABA<sub>A</sub> receptor-positive zones identified as striosomes could appear in a surround of severe loss of GABA<sub>A</sub> receptor immunostaining, yet the preserved striosomes themselves could appear shrunken. Thus, for both the cases identified as predominantly striosome-loss cases or as predominantly matrix-loss cases, there was a spectrum of loss.

The cases designated as mixed-loss cases showed a major and extensive loss of GABA<sub>A</sub> receptor staining in both striosome and matrix compartments (Figs 1D, 2D and 6F) and a corresponding major loss of both calbindin (Fig. 2E) and enkephalin (Fig. 2F) staining in adjacent sections. Figure 6J–L illustrates these patterns at high magnification. Often there was still a remnant of neurochemical staining in the matrix compartment (Figs 1D, 2D–F and 6J–L).

In all of these cases, the staining showed a dorsal-to-ventral gradient of neurochemical change in the dorsal striatum, with relative sparing of the ventral striatum (Figs 1–5) as documented by previous investigators in the Huntington’s disease brain (Vonsattel et al., 1985, 1997; Hedreen and Folstein, 1995; Vonsattel and DiFiglia, 1998; Gunterkunst et al., 2002). We focused on the dorsal striatum (caudate nucleus and putamen) for our analysis. Within the

**Fig. 4** Comparison of the pattern of GABA<sub>A</sub> receptor β<sub>2,3</sub> subunit immunostaining and enkephalin immunostaining in serial sections from matrix-loss Huntington’s disease cases HC78 (grade 3) and HC75 (grade 2). In each case there is pronounced loss of GABA<sub>A</sub> receptor immunostaining in the matrix compartment with a distinctive pattern of densely immunostained zones set against a background of very low receptor immunostaining (A and E). The zones of dense receptor immunostaining (A, C and G) correspond to striosomes visible in the adjoining sections immunostained for enkephalin (B, D and F; H; examples indicated by asterisks). Outlined regions in A, B, E and F are shown at higher magnification in C, D, G and H. Scale bars in B and F = 5 mm, in D and H = 1 mm.
We compared nearby regions in the striosome and matrix compartments in an attempt to take account of the gradients of loss visible in the GABA$_A$, enkephalin and calbindin stains.

Of the 35 Huntington’s disease brains analysed, over two-thirds (25) had particularly pronounced loss in either the striosome compartment or the matrix compartment of the striatum as judged by differential compartmental loss of GABA$_A$ receptor immunostaining at the striatal levels examined. Fourteen cases showed predominant loss in striosomes, 11 cases showed principal loss in the matrix and 10 cases exhibited major loss of GABA$_A$ receptor immunostaining in both the striosome and matrix compartments (Table 1).

To determine whether the patterns of loss that we observed with the GABA$_A$ receptor immunostaining corresponded to patterns of striatal cell loss, we stained adjoining sections from the cases with neuronal N antiserum to mark neuronal perikarya. The stains proved fickle. However, in five cases (two striosome loss, one matrix loss and two mixed loss, spanning Vonsattel grades 1–3) the stains were successful, and there was a clear correspondence between loss of GABA$_A$ receptor immunostaining and loss of NeuN staining (Fig. 7). This evidence suggests that the GABA$_A$ receptor losses we observed did indicate cellular neurodegeneration.

**Relation between clinical symptoms and differential compartmental loss in the striatum**

We found marked clinical heterogeneity in the symptoms expressed by the Huntington’s disease patients both at the point of symptom onset and at clinical end stage. Of the
33 patients with clinical data for symptom onset, 7 had more pronounced mood symptoms than motor symptoms at clinical onset (a >10-point difference in Motor and Mood Indices of the Clinical Huntington’s Disease Questionnaire, Table 4 and Supplementary Table 1 in *Brain* online), 7 had more pronounced motor symptoms than mood symptoms (again, a >10-point difference in Motor and Mood Indices) and 19 had similar levels of symptom severity in both domains. At clinical end stage, all patients had marked motor dysfunction, but there was considerable variability in the level of mood dysfunction, with 12 patients scoring between 0 and 10 on the Mood Index, 8 patients scoring between 11 and 20 and 15 patients with scores >20. To test whether differential compartmental loss of GABA<sub>α</sub> receptor immunostaining was related to the different symptom subtypes exhibited by the patients, the blinding of the clinical and anatomical assessments was broken, and we compared the anatomical results for each case with the indices of motor dysfunction and mood dysfunction for the case both at clinical onset and at end stage.

The results for the striosome-loss cases were clear-cut. Mood dysfunction values were significantly higher in the

---

**Fig. 6** High magnification photomicrographs illustrating the patterns of GABA<sub>α</sub> β3 subunit receptor immunostaining (A, D, G and J), calbindin immunostaining (B, E, H and K) and enkephalin immunostaining (C, F, I and L) in closely spaced sections from a normal case H265 (A, B and C) and from three Huntington’s disease cases (HC79, HC87, HC65) illustrated at lower magnification in Figs 1–3 (see regions outlined in Figs 2 and 3). In normal case H265 (A–C), the zones of slightly enhanced GABA<sub>α</sub> receptor immunostaining (A, example at asterisk) are aligned with striosomes identified in the adjoining sections by low calbindin immunostaining (B) and high enkephalin immunostaining (C). Case HC79 (D–F) shows a major loss of GABA<sub>α</sub> receptor immunostaining in the striosomal compartment. Enkephalin immunostaining (F) is also severely depleted, whereas calbindin staining is preserved in the matrix compartment (E). Case HC87 (G–I) shows severe loss of GABA<sub>α</sub> receptor immunostaining in the matrix compartment with relative preservation of striosomes identified by comparison with enkephalin-rich zones (I). Case HC65 (J–L) illustrates major loss of GABA<sub>α</sub> receptor immunostaining (J) as well as loss of the other neurochemical markers (K and L) in both striosome and matrix compartments. Asterisks indicate examples of striosomes. Scale bars = 1 mm.
immunostaining labelling neurons and neuropil expressing the corresponding to striosomes. (GABA<sub>A</sub> receptor immunostaining in the Huntington's disease case boundaries. It may be noted that the patchy zones of depleted immunostaining, differentially labelling striosomes or their depleted from the striosomes (correspond to calbindin-poor striosomes. Enkephalin has also been zones of depleted NeuN immunostaining, and these zones shown in E sections through the normal striatum. (immunostaining. (closely matches the pattern of cell loss identified by NeuN patterns in serial sections from the caudate nucleus of case HC65, illustrating regions of severely reduced GABA<sub>A</sub> receptor immunostaining corresponding to striosomes. (A and E) GABA<sub>A</sub> receptor immunostaining labelling neurons and neuropil expressing the β<sub>2,3</sub> subunit; (B and F) neuronal N immunostaining, which labels neurons; (C and G) calbindin immunostaining, differentially labelling the matrix compartment; and (D and H) enkephalin immunostaining, differentially labelling striosomes or their boundaries. It may be noted that the patchy zones of depleted GABA<sub>A</sub> receptor immunostaining in the Huntington’s disease case shown in E (example at asterisk) correspond to circumscribed zones of depleted NeuN immunostaining, and these zones correspond to calbindin-poor striosomes. Enkephalin has also been depleted from the striosomes (G). Scale bars = 1 mm.

striosome-loss group than in the matrix-loss group (Fig. 8), and this was true both at clinical onset \( P(U = 48; N1 = 23, N2 = 10) = 0.008 \). This finding held for clinical assessment near death when a directional test was used \( P(U = 81.5; N1 = 14, N2 = 11) = 0.036 \), one-tailed. Given the consistency of this finding, with all four statistical tests significant, there is only an extremely low chance that this represents a Type 1 error.

For the 33 cases with mood scores available for both time periods, we next asked whether individuals with high mood disturbance early in the disease corresponded to those who had high levels of mood disturbance late in the disease. We found that this was the general pattern, but the correlation was not significant, \( \rho = 0.258, P = 0.147 \). Eleven of the 12 cases with the highest mood scores at clinical onset (at or above the 75th percentile) had significant striosomal loss of staining. The mood scores of 7 of these 11 striosome-loss cases were among the 12 cases with the highest (≥75th percentile) mood scores at end stage (all of whom had significant striosomal loss). Two more of the 11 cases had scores between the 50th and 75th percentiles at end stage. The five additional cases with mood scores that were ≥75th percentile at clinical end stage all had pronounced striosome damage, and all had mood scores that increased substantially between the two clinical time points. Of these, two had risen from the 64th percentile to 89th and 94th percentiles and three cases had shown more dramatic increases in mood scores over that time (from 18th and 30th percentiles to ≥89th percentiles). The substantial elevation of mood scores in these latter three cases strongly influenced the size of the correlation for the entire sample; without these cases, the correlation between mood scores at clinical onset and end stage was significant, \( \rho = 0.440, P = 0.015 \).

In contrast to this significant relation between mood dysfunction and striosomal abnormality, we found no evidence of such a relation between predominant abnormal GABA<sub>A</sub> immunostaining in the matrix and the relative severity of overall motor symptomatology at clinical onset \( P = 0.534 \) or at end stage \( P = 0.459 \) (Fig. 8). The relative lack of significant differences in motor dysfunction scores held also for comparisons among all cases exhibiting matrix damage (matrix and mixed groups) and cases having relatively preserved matrix (striosome-loss group), \( P > 0.42 \). As voluntary and involuntary motor symptoms have a distinct clinical course reflecting dysfunction of different neural circuitry, we subdivided motor symptoms into these two components. Cases with matrix loss (matrix and mixed groups) at end stage tended to have higher voluntary motor impairment than cases having relatively preserved matrix, but the difference did not reach significance \( P = 0.069 \).

Relation between clinical symptoms and disease severity
We found a significant correlation for the 35 Huntington’s disease cases analysed here between CAG repeat length and
age of clinical onset ($r = -0.58$, $P < 0.01$), age of death ($r = -0.69$, $P < 0.01$) and Vonsattel pathological grade ($r = 0.67$, $P < 0.01$), consistent with previous findings (Andrew et al., 1993; Illariohnkin et al., 1994; Claes et al., 1995; Furtado et al., 1996). A MANOVA comparing the striosome-loss, matrix-loss and mixed-loss groups on these four variables (CAG repeat length, age of clinical onset, age of death and grade of neuropathology) was significant [Wilkes’ lambda, $F(8,56) = 3.50, P = 0.002$]. Subsequent univariate ANOVAs demonstrated significant differences between the groups for each of the four variables. The striosome-loss group was characterized by lower mean CAG repeat lengths than those of the matrix-loss group ($P = 0.001$) and by lower neuropathological grades than those of either the matrix-loss group ($P < 0.001$) or the mixed-loss group ($P = 0.004$) (Fig. 9 and Table 5). The striosome-loss group also had a later average age of clinical onset ($P = 0.001$) and a later age of death ($P = 0.003$) than the matrix-loss group (Table 5). Disease duration, however, did not differ among the three groups, $F(2,32) = 0.76$, $P = 0.48$. Thus, striosome-predominant neuronal loss in patients was associated both with mood dysfunction and with a milder disease course.

We considered an alternative interpretation, namely, that members of the striosome-loss group were at an earlier stage of disease progression and had died at an earlier clinical stage of the disease than the matrix-loss group. Our evidence...
mood impairment across the two time periods was directly
influenced by CAG repeat length, age and striosome loss. We
used partial correlations between age of onset and age of
death to determine whether the average level of mood
impairment in every case, but in five cases in which we could we found
the zones of major loss of GABA_A receptor staining in the
striatum was characteristic of patients exhibiting prominent
mood dysfunction, and among the patients with high
mood-disorder ratings, pronounced GABA_A receptor loss in
the striosomal compartment was present in most cases. The
association between striosomal abnormality and mood dys-
function held both for early and late-stage symptom assess-
ments, and as a group, the striosome-loss cases tended to
have later ages at clinical onset and at end stage and lower
disease grade and CAG repeat length than did the other cases
studied. Thus patients with prominent mood disorder and
striosome loss may represent a clinical subgroup within the
spectrum of patients suffering from Huntington’s disease.

The general implication of our findings is that the expanded
CAG sequence in the HD gene can affect different functional
subsystems in the forebrain to different degrees.

Our anatomical findings are based on using the expression
of immunohistochemically detectable GABA_A receptors
in relation to striatal compartment themselves identified
by immunohistochemistry. The B_2,3 subunit antibody that
we used is specifically expressed by the medium spiny projec-
tion neurons and the glutamate decarboxylase-positive,
parvalbumin-positive and calretinin-positive classes of
striatal interneuron, which are the classes of neuron
known to be vulnerable in Huntington’s disease
(Waldvogel et al., 1999). We were unable to test whether
the zones of major loss of GABA_A receptor staining in the
Huntington’s disease cases corresponded to zones of cell loss
in every case, but in five cases in which we could we found
clear correspondences between zones of severe loss of
GABA_A receptor immunostaining and zones of severe loss of
NeuN immunostaining. These results suggest that the
patterns of pronounced GABA_A receptor loss correspond

did not support this view. The striosome-loss group had a
significantly later age of death than the matrix-loss group,
and yet did not differ in years of disease duration. This
pattern suggests that the striosome-loss group experienced a
milder disease course than patients in the matrix-loss group,
and that they did not die at an unusually early age, at an
earlier stage of the disease. This observation is supported by
analyses of the causes of death in the different
groups (Table 1). Individuals in the striosome-loss group
were no more likely to have died precipitously from causes
unrelated to end-stage Huntington’s disease than individuals
in the matrix-loss group (Fisher’s exact test, P = 0.656).
Similarly, when all individuals with striosome-loss were
combined, they were not, on average, more likely to have
died precipitously than members of the matrix-loss group
(χ² = 0.031, P = 0.861).

To address the issue of whether the greater mood
dysfunction in Huntington’s disease cases with marked
striosome damage was related to the differential striosome
loss found in these cases or to their lower CAG repeat
lengths, we calculated partial correlations between CAG
repeat length and Mood Indices at clinical onset and at end
stage, controlling for compartment loss (cases with
striosome loss and cases with matrix loss). CAG repeat
length was not significantly correlated with Mood Index
scores at either time-period (r = -0.09, P = 0.65 for clinical
onset, r = -0.18, P = 0.34 for end stage). Similarly, we tested
for the possibility that the greater mood dysfunction in
Huntington’s disease cases with marked striosome damage
was related to the older age of this group by calculating
partial correlations between age of onset and age of death
and the Mood Indices at each time point, controlling for
compartment loss. None of these correlations was significant
(P > 0.5). We further performed a multiple regression
analysis to determine whether the average level of mood
impairment across the two time periods was directly
influenced by CAG repeat length, age and striosome loss.
The regression model using CAG repeat length, age and
compartment groups as predictors of mood impairment was
significant, F(3, 28) = 4.65, P = 0.009. Compartment loss
(cases with predominant striosome loss or with pre-
dominant matrix loss only) was a significant predictor of
average level of mood impairment (beta, standardized =
-0.47, P = 0.02), but neither CAG repeat length (beta,
standardized = -0.29, P = 0.17) nor age of onset (beta,
standardized = -0.21, P = 0.29) significantly predicted
mood. Thus loss of striosomes in Huntington’s disease cases
was significantly associated with mood dysfunction, whereas
CAG repeat length and age were not.

Discussion

Our findings suggest that mood dysfunction in Huntington’s
disease is associated with pronounced abnormality of
striosome-based pathways in the basal ganglia. Loss of
GABA_A receptors in the striosomal compartment of the
striatum was characteristic of patients exhibiting prominent
mood dysfunction, and among the patients with high
mood-disorder ratings, pronounced GABA_A receptor loss in
the striosomal compartment was present in most cases. The
association between striosomal abnormality and mood dys-
function held both for early and late-stage symptom assess-
ments, and as a group, the striosome-loss cases tended to
have later ages at clinical onset and at end stage and lower
disease grade and CAG repeat length than did the other cases
studied. Thus patients with prominent mood disorder and
striosome loss may represent a clinical subgroup within the
spectrum of patients suffering from Huntington’s disease.

The general implication of our findings is that the expanded
CAG sequence in the HD gene can affect different functional
subsystems in the forebrain to different degrees.

Our anatomical findings are based on using the expression
of immunohistochemically detectable GABA_A receptors
in relation to striatal compartment themselves identified
by immunohistochemistry. The B_2,3 subunit antibody that
we used is specifically expressed by the medium spiny projec-
tion neurons and the glutamate decarboxylase-positive,
parvalbumin-positive and calretinin-positive classes of
striatal interneuron, which are the classes of neuron
known to be vulnerable in Huntington’s disease
(Waldvogel et al., 1999). We were unable to test whether
the zones of major loss of GABA_A receptor staining in the
Huntington’s disease cases corresponded to zones of cell loss
in every case, but in five cases in which we could we found
clear correspondences between zones of severe loss of
GABA_A receptor immunostaining and zones of severe loss of
NeuN immunostaining. These results suggest that the
patterns of pronounced GABA_A receptor loss correspond

Table 5 Characteristics of Huntington’s disease cases: total cases, striosome, matrix and mixed groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total cases, n = 35</th>
<th>Striosome cases, n = 14</th>
<th>Matrix cases, n = 11</th>
<th>Mixed cases, n = 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>15 F/20 M</td>
<td>5 F/9 M</td>
<td>5 F/6 M</td>
<td>5 F/5 M</td>
</tr>
<tr>
<td>Age of clinical onset, range</td>
<td>39.2 (13.07) 15–76</td>
<td>47.0 (13.90) 17–76</td>
<td>30.64 (9.22) 15–44</td>
<td>37.7 (9.20) 18–53</td>
</tr>
<tr>
<td>CAG repeat length, range</td>
<td>60.31 (12.14) 39–87</td>
<td>66.51 (11.74) 42–87</td>
<td>52.27 (9.22) 39–63</td>
<td>60.50 (11.15) 45–82</td>
</tr>
<tr>
<td>Neuropathology grade, range</td>
<td>44.24 (3.26) 40–52</td>
<td>42.36 (1.50) 40–46</td>
<td>46.64 (3.64) 42–52</td>
<td>44.22 (3.07) 41–51</td>
</tr>
</tbody>
</table>

Group values represent means, with standard deviations in parentheses. Striosome-loss cases have damage mainly in the striosome compartment. Matrix-loss cases have damage mainly in the matrix compartment. Mixed-loss cases have damage in both the striosome and the matrix compartments.
to patterns of pronounced neuronal loss. Even with the NeuN immunostains, however, as with the GABA_A immunostains, our analysis detected severe loss and would not have detected grades of partial cell loss or, with the GABA_A immunostains, differential loss of particular types of GABA_A immunopositive neurons.

Our anatomical findings are also qualified by other limitations imposed by the human brain material: the classifications we made of striatal compartment and GABA_A receptor loss were made with respect to a limited part of the caudate nucleus and putamen (generally mid-anterior levels), and were qualitative not quantitative, owing to the lack of total striatal and compartmental volumetric measurements and the gradients of immunostaining evident even in the normal brains. We did not analyse the ventral striatum, which tends to be relatively spared in Huntington’s disease, nor other brain regions, including the neocortex, which is strongly affected. Nevertheless, our findings directly demonstrate that within the Huntington’s disease striatum, predominant GABA_A receptor loss can be localized mainly to either the striosomal or the matrix compartment, and that these compartmental patterns of abnormality are related to different patterns of behavioural symptomatology expressed by Huntington’s disease patients.

This result should help resolve the disparities among previous studies. Our analysis suggests that either compartment can suffer predominant neurochemical loss. In the first detailed study of cell death and astrocytosis in relation to striatal compartmentalization, Hedreen and Folstein (1995) suggested that striatal degeneration in Huntington’s disease begins with cell death in striosomes, followed by a second wave of neuronal degeneration in the matrix. Others, however, have suggested that degeneration in the matrix is the principal deficit (Olsen et al., 1986; Ferrante et al., 1987; Seto-Ohshima et al., 1988; Faull et al., 1993). In our case sample, we found, in agreement with Hedreen and Folstein, that Huntington’s disease cases with predominant striosomal defects typically had low neuropathological grades of the disease (grades of 0, 1 or 2). In addition to cases with predominant striosome loss, however, our material included some Huntington’s cases with severe GABA_A receptor loss in the matrix and relative sparing of striosomes in the same striatal regions, even in higher grade cases. These cases of predominant matrix loss suggest that either loss of striosomes does not always precede loss of matrix, or, if there is a fixed temporal ordering of compartmental deficit, with striosome loss occurring first, the eventual pattern of degeneration can nevertheless yield a severely degenerated matrix with relatively preserved striosomes.

We did not address directly the issue of disease progression, but our findings suggest that striosome-loss cases had the same average disease duration as the matrix-loss cases, and that they had a later age of onset and died later in life than the matrix-loss cases. These data suggest that the individuals with predominant striosome loss did not die at an earlier stage of their disease progression than did the other patients. What did distinguish the striosome loss from the matrix-loss cases in our sample is that cases with marked striosome-loss cases in our sample is that cases with marked striosome loss exhibited more severe mood disturbances and a milder disease course.

We found no strong link between differential abnormality in the matrix compartment and general motor dysfunction. There was a trend towards higher end-stage scores for voluntary motor dysfunction in the matrix-loss cases than in cases of striosome loss. The lack of a clear association between matrix abnormality and general motor function could reflect large-scale degeneration of other brain regions in these cases, decreasing the amount of variance accounted for by striatal damage, or neurochemical heterogeneity in the matrix itself. The matrix has a modular input–output architecture that is not visible in conventional stains, and this striatal compartment receives a wide variety of cortical inputs from regions of association cortex as well as motor and premotor cortex (Flaherty and Graybiel, 1994; Eilen and Graybiel, 1995). Differential loss of subsets of input–output connections in the matrix would not have been detectable in our analysis. We also did not include an analysis of cognitive deficits, known to occur in many Huntington’s disease patients, but for which we could not gather reliable retrospective data. These symptoms might also have shown different associations with the anatomically selective patterns of striatal abnormality or have influenced the associations that we did observe.

Our findings provide the first demonstration of a relationship between the striosome compartment of the striatum and mood disturbance in humans. Our definition of mood disorder was broad. It included symptoms of depression, anxiety and irritability, as well as types of compulsive behaviour and repetitive activity. In experimental animals, the striosomal compartment has been singled out as having particularly strong connections with regions of the limbic forebrain including the anterior cingulate cortex, the caudal orbitofrontal cortex and the basolateral amygdala (Eilen and Graybiel, 1995), all of which have been implicated in mood and disorders of mood and emotional function in humans and in obsessive-compulsive spectrum disorders (Graybiel and Rauch, 2000; Paus, 2001; Cardinal et al., 2002; Rolls, 2004; Mayberg et al., 2005; Phelps, 2006). The functions of the striosomal system are unknown, but in non-human primates and rodents, differential activation of at least part of this striatal system occurs in response to drug treatments that induce repetitive stereotyped behaviour suggestive of compulsive activity (Canales and Graybiel, 2000; Saka et al., 2004), and striosomes have been linked to motivation-based behaviours in these species (Aosaki et al., 1995; White and Hiroi, 1998).

Our findings raise the possibility that striosomes modulate affective state in the human by way of striosome-linked corticobasal ganglia circuits.

In the Huntington’s disease cases with pronounced striosome loss, CAG repeat lengths were on average lower than those in cases with matrix loss or mixed compartmental
loss of GABA$_{A}$ receptor immunostaining, and cases with prominent mood dysfunction tended to have lower CAG repeat lengths. Our analyses indicate that the level of mood impairment was related to the striosome loss rather than to CAG repeat length. Previous studies documenting clinical variability in Huntington’s disease patients have also failed to find a clear association between CAG repeat length and symptom subtype (Claes et al., 1995; Zappacosta et al., 1996). The critical contribution of our study is the demonstration that Huntington’s disease is a heterogeneous disease both in its symptom profile and in its pattern of compartmental abnormality in the striatum, and that these two phenotypes may be related. This heterogeneity is likely to result from variable influences of modifier genes and other epigenetic effects. Microrarray analysis of tissue from the caudate nucleus of 34 Grade 0–2 Huntington’s disease cases, including cases from this study, suggests that 20% of striatal mRNAs are differentially expressed in the disease striatum relative to the striatum of controls (Hodges et al., 2006). Environmental factors can influence the rate of symptom progression (van Dellen et al., 2000) and neurodegenerative changes (Glass et al., 2004) in animal models of Huntington’s disease. Thus the CAG sequence in the HD gene is critical in determining Huntington’s disease risk, but it is not the sole determinant of symptom profile. The striosome–mood disorder relationship we report here suggests that forebrain-subsystem specificity may be key to understanding the differential symptom phenotypes and patterns of neurodegeneration in Huntington’s disease.

Supplementary material
Supplementary data are available at Brain Online.

Acknowledgements
We express our appreciation to the Huntington’s families in New Zealand and to Beth Gordon (Huntington’s Disease Association, Auckland) for their generous and invaluable assistance during the course of the study, to the Neurological Foundation of New Zealand Human Brain Bank for providing the human brain tissue, to Henry F. Hall of the Massachusetts Institute of Technology (MIT) for his help and to Drs Ruth Bodner and J. Michael Andresen of MIT for discussing the data. This study was supported by grants from the Health Research Council of New Zealand, the Neurological Foundation of New Zealand, the University of Auckland Research Committee, the Matthew Oswin Memorial Trust and the US National Institute of Neurological Disorders and Stroke (Javits award NS538372). Funding to pay the open access charges for this article was provided by the Health Research Council of New Zealand.

References


Glass M, van Dellen A, Blakemore C, Hannan AJ, Faull RL. Delayed onset of Huntington’s disease in mice in an enriched environment correlates...
Striosomes and mood in Huntington's disease

with delayed loss of cannabinoid CB1 receptors. Neuroscience 2004; 123: 207–12.


