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Altered neurotransmitter receptor expression in transgenic mouse models of Huntington's disease

Jang-Ho J. Cha[1*], Ariel S. Frey[1], Stephen A. Alsdorf[1], Julie A. Kerner[1], Christoph M. Kosinski[1], Laura Mangiarini[2], John B. Penney Jr[1], Stephen W. Davies[3], Gillian P. Bates[2] and Anne B. Young[1]

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Alterations in neurotransmitter receptors are a pathological hallmark of the neurodegeneration seen in Huntington's disease (HD). However, the significance of these alterations has been uncertain, possibly reflecting simply the loss of brain cells. It is not known for certain whether the alteration of neurotransmitter receptors occurs before the onset of symptoms in human HD. Recently we developed transgenic mice that contain a portion of a human *HD* gene and develop a progressive abnormal neurological phenotype. Neurotransmitter receptors that are altered in HD (receptors for glutamate, dopamine, acetylcholine and adenosine) are decreased in the brain of transgenic mice, in some cases before the onset of behavioural or motor symptoms. In transgenic mice, neurotransmitter receptor alterations occur before neuronal death. Further, receptor alterations are selective in that certain receptors, namely *N*-methyl-D-aspartate and γ-aminobutyric acid receptors, are unaltered. Finally, receptor decreases are preceded by selective decreases in the corresponding mRNA species, suggesting the altered transcription of specific genes. These results suggest that (i) receptor decreases precede, and therefore might contribute to, the development of clinical symptoms, and (ii) altered transcription of specific genes might be a key pathological mechanism in HD.

Keywords: Huntington's disease; transgenic mouse; glutamate; receptor; hybridization *in situ*; striatum

1. INTRODUCTION

Huntington's disease (HD) is an autosomal dominantly inherited neurodegenerative disease caused by an expansion of a CAG trinucleotide repeat motif within the *huntingtin* gene (Huntington's Disease Collaborative Research Group 1993). HD is therefore a member of a newly defined class of neurodegenerative diseases characterized by CAG expansions, CAG being the codon for glutamine. Hence CAG diseases are also known as polyglutamine diseases (Paulson & Fischbeck 1996; Perutz 1996; Reddy & Housman 1997). Pathologically, HD is characterized by neurodegeneration of the striatum and the deep layers of cerebral cortex (Vonsattel *et al*. 1985; de la Monte *et al*. 1988). Many changes in the neurotransmitter receptor have been identified in the striatum of patients with HD, including decreases in glutamate, dopamine, γ-aminobutyric acid (GABA), muscarinic cholinergic and adenosine receptors (Reisine *et al*. 1977; London *et al*. 1981; Penney & Young 1982; Van Ness *et al*. 1982; Walker *et al*. 1984; Young *et al*. 1988; Dure *et al*. 1991; Martinez-Mir *et al*. 1991; Richfield *et al*. 1991; Faull *et al*. 1993). Alteration of neurotransmitter receptors, particularly of glutamate receptors, has been postulated to have an aetiological role in the striatal neurodegeneration seen in HD. According to one school of thought, neuronal damage is initiated through the abnormal activation of striatal neurons. Indeed, intrastriatal administration of exogenous glutamate receptor agonists, particularly those agents activating the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptor, reproduces the neuropathological features of HD (Beal *et al*. 1986; DiFiglia 1990; Albin & Greenamyre 1992).

An alternative hypothesis is that receptor alterations observed in post-mortem HD brains are simply a reflection of dying or dead neurons. That is, when neurons die, one can logically expect a resultant decrease in the number of neurotransmitter receptors. For example, the well-described decrease in GABA levels in HD striatum reflects the fact that, of all striatal neurons, the GABA-containing striatal projection neurons are preferentially lost early in the course of HD. Measuring neurotransmitter receptor levels in post-mortem HD brains, then, is akin to examining the ashes of a house that has burnt to the ground. Although one might be able to conclude that there has been a fire, it might be extremely difficult to pinpoint where the fire began and how it spread to involve other structures. If effective therapies for HD are

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ever to become a reality, a thorough understanding of the molecular mechanisms involved in the pathogenesis of neurodegeneration is essential. That is, to intervene in the pathogenesis of neuronal damage it is essential to delineate exactly the steps through which such damage occurs, and to identify those changes that are merely epiphenomena.

Although post-mortem studies of neurotransmitter receptors are somewhat confounded by neuronal loss, recent findings indicate that the alteration of neurotransmitter receptors might occur before the onset of symptoms. In presymptomatic patients with HD, dopamine receptors were decreased, and D_1 and D_2 receptor mRNA species have been found to be decreased in a grade 0 post-mortem HD brain (Antonini *et al*. 1996; Weeks *et al*. 1996; Augood *et al*. 1997). Similarly, evidence exists that there are early decreases in the neuropeptides enkephalin and substance P, which are used by striatal projection neurons (Albin *et al*. 1990; Augood *et al*. 1996). Again, ante-mortem studies of presymptomatic HD patients are quite few; one cannot exclude the possibility that early neurotransmitter receptor alterations reflect a mild degree of neuronal loss.

We have recently developed transgenic mice that contain a portion of an abnormal human HD chromosome (Mangiarini *et al*. 1996). Several of these mouse lines, named R6/1, R6/2 and R6/5, develop a progressively abnormal neurological phenotype. Although they seem normal at birth, these transgenic mice gradually become more and more uncoordinated, develop abnormal movements and lose body weight. A striking feature of these mice is the observation of novel structures that appear in the nucleus, dubbed neuronal intranuclear inclusions (NII) (Davies *et al*. 1997). Abnormal inclusions have also been found in human HD necropsy material (Roizin *et al*. 1979; DiFiglia *et al*. 1997) and have been postulated to have a role in the pathogenesis of HD and other CAG repeat diseases (Ross 1997; Davies *et al*. 1998). The ultimate significance of abnormal protein aggregation has been hotly debated and remains to be settled (Perutz *et al*. 1994; Lansbury 1997).

We have investigated neurotransmitter levels in various lines of transgenic mice containing fragments of the human HD gene. Specifically, we have investigated neurotransmitter receptors in mouse lines with abnormal neurological phenotypes as well as other lines that seem neurologically normal. Where we have found altered neurotransmitter receptors, we have examined mice of various ages to determine whether receptor changes precede or follow the onset of clinical symptoms. To measure receptors at the protein level, we have used receptor binding autoradiography and immunoblotting. We have also used hybridization *in situ* to assess the role of mRNA in receptor protein alteration.

2. MATERIALS AND METHODS

(a) *Transgenic mice*

The mice used in the current study have been described previously (Mangiarini *et al*. 1996; Davies *et al*. 1997; Bertaux *et al*. 1998). In brief, several strains of transgenic mice carrying a 1.9 kb fragment of the 5′ end of the human *HD* gene have been generated on a CBAxC57BL/6 background. Lines R6/1, R6/2 and R6/5 all develop an abnormal neurological phenotype. Although heterozygotes of lines R6/1 and R6/2 demonstrate an abnormal neurological phenotype, R6/5 heterozygotes seem normal; R6/5 homozygotes were used in the current study. R6/2 mice demonstrate symptoms at *ca*. 9–11 weeks of age, whereas R6/1 mice demonstrate symptoms at 15–21 weeks and R6/5 homozygotes develop abnormal symptoms at approximately nine months of age. Lines R6/1, R6/2 and R6/5 all carry CAG repeat numbers in excess of 100. In contrast, HDex6 and HDex27 are transgenic mouse lines that contain a fragment of the human *HD* gene carrying only 18 CAG repeats (within the 'normal' human range of CAG repeats).

(b) *Receptor binding studies*

All studies were performed on coded samples by experimenters blinded to the genotype status of the animals. For quantitative glutamate receptor autoradiography, mouse brains were frozen rapidly in liquid isopentane and kept at −70 °C until use. Sections (12 μm) containing striatum and cortex were thaw-mounted on polylysine-coated glass slides. Slide mounted sections were thawed at room temperature for 1 h before use. For glutamate and GABA receptor studies, slides were prewashed in assay buffer for 30 min at 4 °C, then dried under a stream of cool air. Slides were then incubated for 45 min in ^{3}H-labelled ligand in the presence or absence of displacers. The conditions are presented in summary form as follows.

Assay: concentration of ^{3}H-labelled ligand (specific radioactivity); assay buffer; displacers present in the incubation assay; non-specific 'blank' condition.

(RS)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors: 20 nM [^{3}H]AMPA (specific radioactivity 52.3 Ci mmol^{-1}); 50 mM Tris–HCl with 2.5 mM $CaCl_2$ and 30 mM KSCN, pH 7.2 (Tris–HCl/$CaCl_2$/KSCN); displacers, none; blank, 1 mM glutamate (Cha *et al*. 1992).

Kainate receptors: 80 nM [^{3}H]kainate (specific radioactivity 58 Ci mmol^{-1}); 50 mM Tris–acetate, pH 7.2; displacers, none; blank, 1 mM kainate (Greenamyre *et al*. 1985).

NMDA receptors: 100 nM [^{3}H]glutamate (specific radioactivity 49 Ci mmol^{-1}); 50 mM Tris–acetate, pH 7.4; displacers, none; blank, 1 mM NMDA (Greenamyre *et al*. 1985).

Group I metabotropic glutamate receptors: 100 nM [^{3}H]glutamate (specific radioactivity 49 Ci mmol^{-1}]); Tris–HCl/$CaCl_2$/KSCN; displacers, 100 μM NMDA and 10 μM AMPA; blank, 2.5 μM quisqualate (Cha *et al*. 1990).

Group II metabotropic glutamate receptors: 100 nM [^{3}H]glutamate (specific radioactivity 49 Ci mmol^{-1}); Tris–HCl/$CaCl_2$/KSCN; displacers, 100 μM NMDA, 10 μM AMPA and 2.5 μM quisqualate; blank, 1 mM glutamate (Catania *et al*. 1993).

$GABA_A$ receptors: 40 nM [^{3}H]GABA (specific radioactivity 29.05 Ci mmol^{-1}); Tris–HCl plus 2.5 mM $CaCl_2$, pH 7.4; displacers, 100 μM baclofen; blank, 100 μM isoguvacine (Chu *et al*. 1990).

$GABA_B$ receptors: 40 nM [^{3}H]GABA (specific radioactivity 29.05 Ci mmol^{-1}); Tris–HCl plus 2.5 mM $CaCl_2$, pH 7.4; displacers, 100 μM isoguvacine; blank, 100 μM baclofen (Chu *et al*. 1990).

All ^{3}H-labelled ligands were obtained from New England Nuclear (Boston, MA, USA). After incubation in ^{3}H-labelled ligand, slides were subjected to three rapid washes in cold buffer, then one rapid wash in glutaraldehyde–acetone (25%, v/v), and dried quickly under a stream of warm air. Slides were apposed to tritium-sensitive film (Hyperfilm ^{3}H; Amersham, Arlington,

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IL, USA) with calibrated radioactive standards and exposed for two to three weeks. Films were developed and analysed with a computer-based image analysis system (M1; Imaging Research, St Catharine's, Ontario, Canada). Image density corresponding to the binding of ^{3}H-labelled ligand was converted to picomoles per milligram of protein by using calibrated radioactive standards; non-specific binding was subtracted.

D_1 and D_2 dopamine receptor assays used a buffer containing 25 mM Tris–HCl, pH 7.5, 100 mM NaCl, 1 mM $MgCl_2$, 1 μM pargyline and 10 mg l^{-1} ascorbate. For D_1 receptors, slides were incubated with 1.65 nM [^{3}H]SCH-23390 (specific radioactivity 70.3 Ci $mmol^{-1}$) for 2.5 h. Non-specific binding was defined in the presence of 1 μM *cis*-flupentixol (Richfield *et al.* 1986). For D_2 receptors, slides were incubated with 180 pM [^{3}H]YM-09151-2 (specific radioactivity 85.5 Ci $mmol^{-1}$) for 3 h. Non-specific binding were defined in the presence of 50 μM dopamine (Cox & Waszczak 1991). For [^{3}H]mazindol binding, slides were prewashed for 5 min in cold buffer containing 50 mM Tris–HCl, 5 mM KCl and 300 mM NaCl, pH 7.9. Slides were then incubated in 6 nM [^{3}H]mazindol (specific radioactivity 24 Ci $mmol^{-1}$) in the presence of 300 nM desipramine for 2.5 h. Non-specific binding was defined in the presence of 10 μM nomifensine (Javitch *et al.* 1983). After incubation in ^{3}H-labelled ligand, slides were rinsed in cold buffer for 10 min, then rinsed quickly in distilled water. Slides were dried under a stream of cool air. Film exposure and analysis were performed as described above.

For muscarinic cholinergic receptors, slides were prewashed twice for 5 min in cold buffer containing 7.75 mM Na_2PO_4, 137 mM NaCl and 2.6 mM KCl. Slides were incubated in 1 nM [^{3}H]QNB (specific radioactivity 49 Ci $mmol^{-1}$) for 3 h (Penney & Young 1982). After incubation, slides were rinsed twice for 5 min in cold buffer, followed by a quick rinse in distilled water. Slides were dried under a stream of cool air. For A2a adenosine receptors, the buffer used was 50 mM Tris–HCl, pH 7.4, containing 10 mM $MgCl_2$. After a preincubation for 30 min at room temperature in buffer containing 2 units ml^{-1} adenosine deaminase, slides were incubated in buffer containing 5 nM [^{3}H]CGS 21680 (specific radioactivity 39.5 Ci $mmol^{-1}$) for 90 min (Jarvis & Williams 1989). Non-specific binding was defined in the presence of 20 μM 2-chloroadenosine. Slides were rinsed for 5 min in ice-cold buffer, then quickly in ice-cold doubly distilled water, and dried rapidly under a stream of warm air. Film exposure and analysis were as described above.

(c) *Immunoblotting*

Frozen mouse brain hemispheres were sonicated in ten volumes of homogenization buffer containing 50 mM Tris–HCl, 10% (v/v) glycerol, 5 mM magnesium acetate and 0.2 mM EDTA, with protease inhibitors (phenylmethanesulphonyl fluoride, leupeptin and aprotinin). Protein concentrations were determined by Bio-Rad protein assay (Bio-Rad, Hercules, CA, USA). With a 96-well manifold, 2.5 μg of protein was loaded into each well and transferred on nitrocellulose membrane (0.45 μm pore size; Bio-Rad). Nitrocellulose membranes were washed in Tris-buffered saline with 0.1% (v/v) Tween 20 (TTBS) (three washes, each for 10 min). Membranes were incubated for 1 h with a 1:2500 dilution of primary antibody. Primary antibodies were directed against mGluR1a (rabbit polyclonal; Chemicon, Temecula, CA, USA), mGluR2/3 (rabbit polyclonal; Chemicon), mGluR5 (rabbit polyclonal; Upstate Biotechnology, Lake Placid, NY, USA), NMDA-R1 (mouse monoclonal; Chemicon) and ubiquitin (rabbit polyclonal; Dako, Glostrup, Denmark). Membranes were washed three times (each for 10 min) in TTBS, and then incubated for 1 h in horseradish peroxidase-conjugated secondary antibody (either goat anti-rabbit or goat anti-mouse) at dilutions of 1:1000 (for mGluR1a and mGluR2/3) or 1:5000 (for ubiquitin, mGluR5 and NMDA-R1). After three 10 min washes in TTBS, immunoreactivity was detected with chemiluminescence (SuperSignal; Pierce, Rockford, IL, USA). The resulting film images were analysed with the M1 image analysis system. Film density readings were measured from four dots from each sample; film background was subtracted. Density readings were normalized to density readings from control mouse brain homogenates. Western blots were performed on control mouse brain homogenates to confirm that the glutamate receptor antibodies employed recognized primarily single bands under the immunoblot conditions employed.

(d) *Hybridization* in situ

RNA probes were generated from PCR templates by using SP6 (for anti-sense probes) or T7 (for sense probes) RNA polymerases in accordance with the manufacturer's instructions (Promega, Madison, WI, USA) (Standaert *et al.* 1996). Slide-mounted brain sections (12 μm) were warmed to room temperature, fixed in 40 g l^{-1} paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, washed in 0.1 M phosphate buffer containing 0.9 g l^{-1} NaCl, pH 7.4 (PBS), acetylated in 0.1 M triethanolamine pH 8.0 with 0.25% (v/v) acetic anhydride, rinsed in PBS, dehydrated through graded ethanol solutions, and delipidated in chloroform. [^{35}S]CTP-labelled sense or anti-sense RNA probes were hybridized in 50% (v/v) formamide, 0.3 M NaCl, 10 mM Tris–HCl, pH 8.0, 1 mM EDTA, 10% (v/v) dextran sulphate, 1 × Denhart's solution, 100 mM dithiothreitol, 1 g l^{-1} SDS, 1 g l^{-1} sodium thiosulphate, 100 μg ml^{-1} salmon sperm DNA, 250 μg ml^{-1} yeast tRNA and 250 μg ml^{-1} yeast total RNA at 50 °C for 4 h. After hybridization, sections were washed in 2 × SSC at room temperature, then in 0.1×SC at 70 °C, treated with RNase A (100 μg ml^{-1} in 0.5 M NaCl, 10 mM Tris–HCl and 1 mM EDTA, pH 7.2) at 37 °C, washed in 0.1 × SSC at 70 °C, and dried in an ethanol series. Slides were apposed to autoradiographic film and developed after two to seven days. Image analysis was performed with the M1 image analysis system. Oligodeoxynucleotides specific for human β-actin were labelled with [^{35}S]dATP (Wüllner *et al.* 1994). Prehybridization was the same as for RNA probes with the exception that chloroform was omitted. [^{35}S]DNA probe was hybridized in 50% (v/v) formamide, 0.3 M NaCl, 10 mM Tris–HCl, pH 8.0, 1 mM EDTA, 10% (v/v) dextran sulphate, 1× Denhart's solution and 100 mM dithiothreitol at 37 °C overnight. After hybridization, sections were washed in decreasing concentrations of SSC and dried in an ethanol series. The slides were then apposed to autoradiographic film for between three days and two weeks and analysed as described above.

3. RESULTS

We studied glutamate receptor binding autoradiography in the brains of symptomatic 12-week-old R6/2 mice (figure 1). All studies were conducted on coded samples such that the investigators were blinded to the genotype of the sample tissues. Ionotropic glutamate receptors (NMDA, AMPA and kainate receptors) showed varying alterations. Although NMDA receptor binding was the same in the brains of transgenic mice as in

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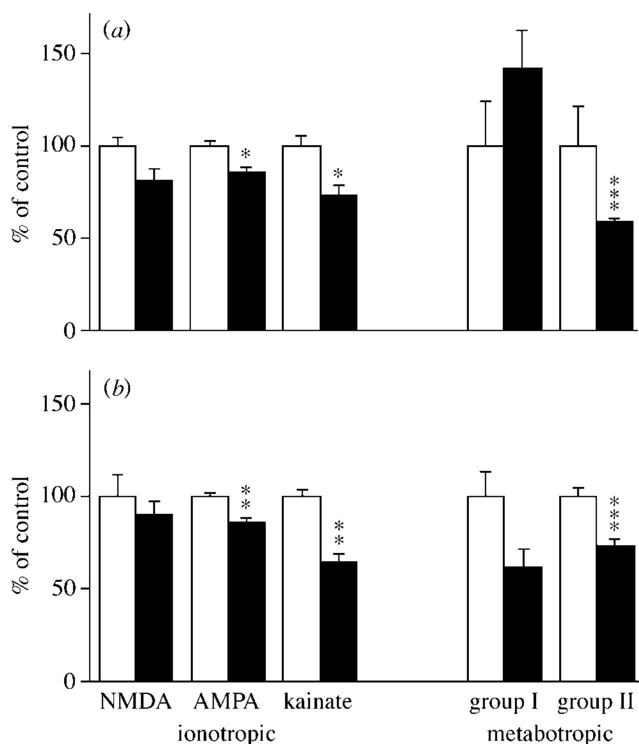

Figure 1. Glutamate receptor binding in 12-week-old control (open bars) and R6/2 transgenic (filled bars) mouse brain: (*a*) cortex; (*b*) striatum. Numbers are percentages of normal mouse binding levels (pmol of ^{3}H-labelled ligand bound per mg of protein, mean ± s.e.m., $n = 12$ animals per group except for kainate receptors, where $n = 6$ animals per group). $^*p < 0.01$; $^{**}p < 0.001$; $^{***}p < 0.0001$.

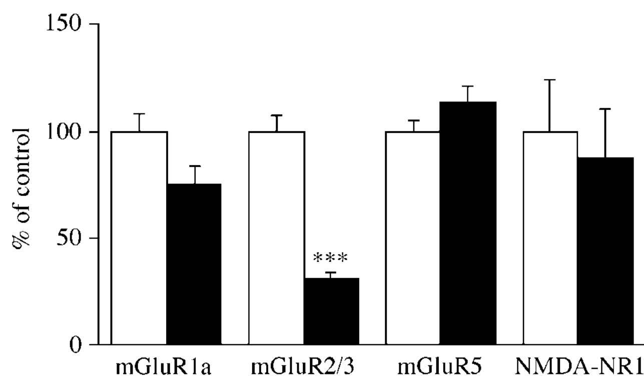

Figure 2. Immunoblots for glutamate receptor proteins in 12-week-old control (open bars) and R6/2 transgenic (filled bars) mouse brain homogenates. Numbers are percentages of normal mouse levels (mean ± s.e.m., $n = 6$ animals per group). $^{***}p < 0.0001$.

normals, AMPA receptors were decreased to 85% of normal, and kainate receptor binding was decreased to 63.5% (striatum) and 72.8% (cortex) of normal. Of the metabotropic glutamate receptors, group I mGluR binding was not statistically different between R6/2 transgenic and control mice. Group II mGluR binding was clearly affected, however, as it decreased to 72.5% (striatum) and 58.7% (cortex) of normal values. Receptor binding alterations were present equally in the striatum and cortex of transgenic R6/2 mice.

Because huntingtin has been postulated to have a role in protein trafficking (DiFiglia *et al.* 1995, 1997; Sapp *et al.* 1997), one possibility is that the decreases in group II mGluR binding were due to abnormal trafficking of receptors, in which case the overall level of protein expression might not be decreased. To investigate this possibility, we performed immunoblotting to determine whether overall protein levels were decreased. Immunoblotting data indicated that not only was group II metabotropic glutamate receptor (mGluR2 and mGluR3) binding decreased, but also the amounts of mGluR2/3 metabotropic receptor protein immunoreactivity (figure 2). There was no decrease in the immunoreactivity for the mGluR5 subtype of group I metabotropic glutamate receptor or for the NMDA-R1 subunit of NMDA receptor. Therefore, the decrease in receptor protein expression was selective for certain receptor subtypes but not all receptors. Decreased mGluR2/3 protein levels were confirmed by using immunohistochemistry with an antibody that recognized both mGluR2 and mGluR3, demonstrating decreased cortical neuropil staining in R6/2 mice. Quantification of confocal laser images of mGluR2/3 immunostaining revealed statistically significant decreases in R6/2 cortex (76.2% of control, $p < 0.0001$) and in striatum (89.3% of control, $p < 0.0001$).

Decreases in receptor protein could result from decreased translation of mRNA into protein or from decreased transcription of genes into mRNA. To differentiate between these possibilities, we performed hybridization analyses *in situ* for glutamate receptor mRNA. In the brains of symptomatic 12-week-old R6/2 mice, there were marked decreases in the expression of mRNA for mGluR1, mGluR2 and mGluR3 (figure 3). Decreased mRNA expression for these receptors was unlikely to represent simply cell loss, as no difference in mRNA levels was measured for mGluR5, NMDA-R1 or β-actin. Therefore, the selectivity of alteration of receptor protein expression was also found at the mRNA level.

Given the selective alterations in glutamate receptors, we investigated other neurotransmitter receptors known to be affected in human HD brain (figures 4 and 5). At 12 weeks, when R6/2 mice are clearly symptomatic, quantitative receptor autoradiography revealed statistically significant decreases in R6/2 striatal receptor binding for [^{3}H]QNB, a ligand for muscarinic acetylcholine receptors (35% decrease), [^{3}H]SCH 23390, a ligand for the D_1 family of dopamine receptors (67% decrease), and [^{3}H]YM-09151-2, a ligand for the D_2 family of dopamine receptors (59% decrease). A decrease in [^{3}H]QNB binding was also observed in the cortex of transgenic animals, indicating that the alterations in receptor binding were not limited to the striatum. There were no significant changes in $GABA_A$ or $GABA_B$ receptors, or in [^{3}H]mazindol binding, a measure of dopamine uptake sites. A2a adenosine receptors, which are present on the striatal projection neurons that degenerate in HD, are also decreased in the striatum of 12-week-old R6/2 transgenic mice (figure 6).

R6/2 mice develop neurological symptoms by 9–11 weeks. The observed changes in neurotransmitter receptors in the brains of 12-week-old mice could therefore be secondary to a generalized pathological process. To delineate the temporal relationship of neurotransmitter

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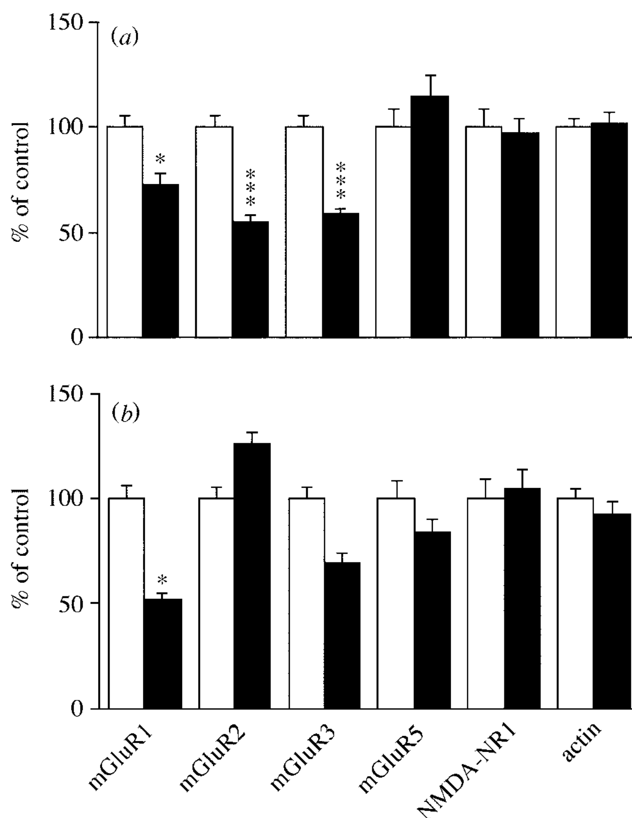

Figure 3. Hybridization *in situ* for glutamate receptors in 12-week-old control (open bars) and R6/2 transgenic (filled bars) mice: (*a*) cortex; (*b*) striatum. Numbers are film intensities expressed as percentages of normal cortex signal. $^{*}p < 0.01$; $^{***}p < 0.0001$.

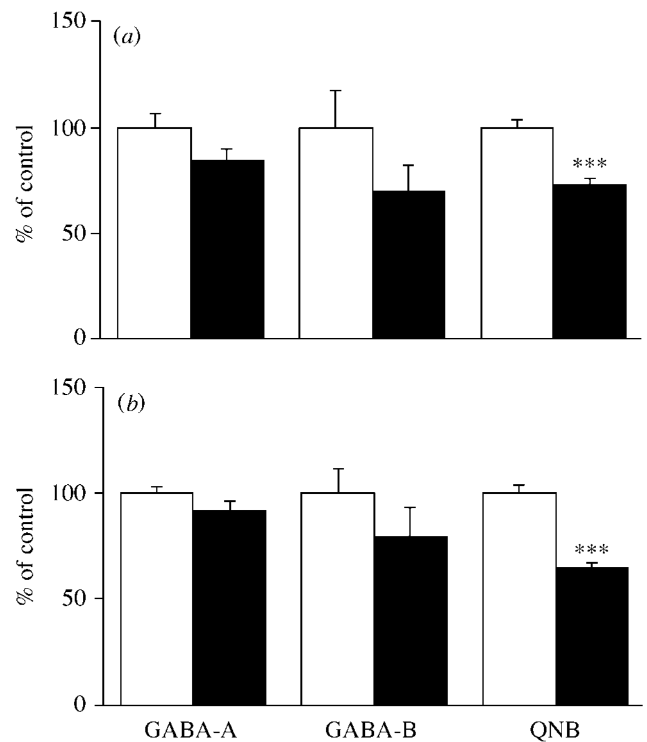

Figure 4. Receptor binding for $GABA_A$, $GABA_B$ and muscarinic acetycholine (labelled with QNB) receptors in 12-week-old control (open bars) and R6/2 transgenic (filled bars) mouse brain: (*a*) cortex; (*b*) striatum. Numbers are percentages of control mouse levels (mean ± s.e.m., $n = 6$ animals per group). $^{***}p < 0.0001$.

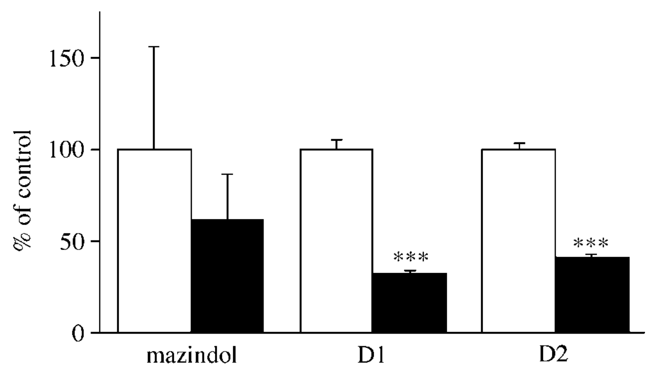

Figure 5. Receptor binding for D_1 dopamine, D_2 dopamine and dopamine uptake sites (labelled with mazindol) in 12-week-old control (open bars) and R6/2 transgenic (filled bars) mouse striatum. Numbers are percentages of control mouse levels (mean ± s.e.m., $n = 6$ animals per group). $^{***}p < 0.0001$.

receptor alteration to the development of symptoms, we performed receptor binding and hybridization *in situ* on a series of mice of different ages, including presymptomatic transgenic mice. Again, all experiments were performed on coded tissue samples by blinded investigators. Both D_1 and D_2 dopamine receptors, assessed by the binding of receptors, were not significantly different from controls at two and four weeks of age, but were significantly decreased at eight and 12 weeks (figures 7 and 8). A2a adenosine receptors were normal at two weeks of age, but significantly decreased by four weeks (figure 9). These results indicate that the lower neurotransmitter receptor levels in the 12-week-old R6/2 mice are not simply the result of abnormal receptor development. Rather, dopamine and adenosine receptors seem to develop normally in R6/2 transgenic mice and then are selectively lost.

Hybridization *in situ* revealed that the D_1 dopamine receptor and A2a adenosine receptor mRNA signal in R6/2 transgenic mouse striatum was statistically decreased by four weeks of age (figure 10). We have previously observed decreases in metabotropic glutamate receptor mRNA in four-week-old and eight-week-old R6/2 mice (Cha *et al.* 1998). Thus, in eight-week-old animals, there are already major alterations in the glutamate, dopamine and adenosine neurotransmitter systems, which have major importance in striatal function. There is also evidence of receptor mRNA expression changes beginning as early as four weeks. It is therefore likely that specific mRNA decreases occur before measurable decreases in receptor protein.

Transgenic animals might manifest phenotypic differences from wild-type mice because of the introduced transgene or because of disruption of endogenous mouse DNA. To confirm that the receptor alterations observed in the R6/2 line were not simply due to a non-specific effect of the transgene, we examined other lines of transgenic mice. Other transgenic mouse lines that demonstrated abnormal neurological phenotypes (R6/1 and R6/5) also demonstrated decreases in D_1 dopamine, D_2 dopamine and A2a adenosine receptor binding, similar to those of the R6/2 line (figure 11). HDex6 and HDex27 are two transgenic lines that contain a human *HD* transgene containing a 'normal' number of CAG repeats. These lines

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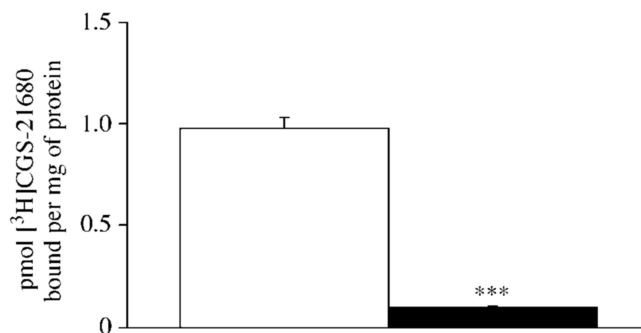

Figure 6. Receptor binding for A2a adenosine receptors in 12-week-old control (open bars) and R6/2 transgenic (filled bars) mouse striatum. Numbers are percentages of control mouse levels (mean ± s.e.m., $n = 6$ animals per group). $^{***}p < 0.0001$.

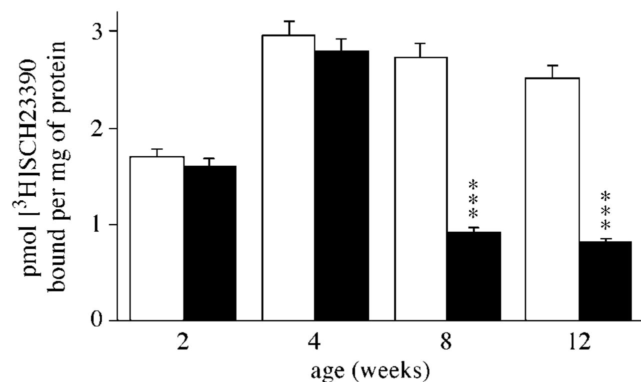

Figure 7. Developmental profile of D_1 receptor binding in control (open bars) and R6/2 transgenic (filled bars) mouse striatum. Numbers are pmol mg^{-1} protein of bound ligand levels (mean ± s.e.m., $n = 6$ animals per group). $^{***}p < 0.0001$.

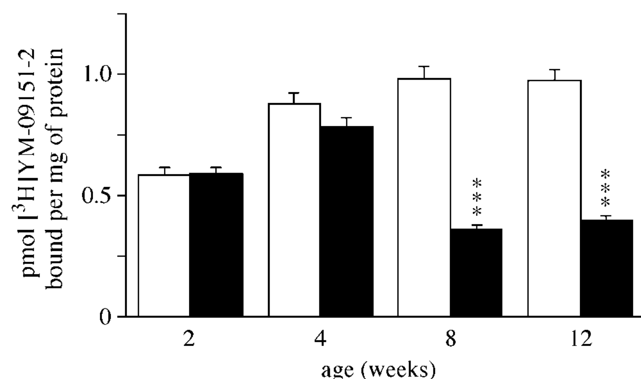

Figure 8. Developmental profile of D_2 receptor binding in control (open bars) and R6/2 transgenic (filled bars) mouse striatum. Numbers are pmol mg^{-1} protein of bound ligand levels (mean ± s.e.m., $n = 6$ animals per group). $^{***}p < 0.0001$.

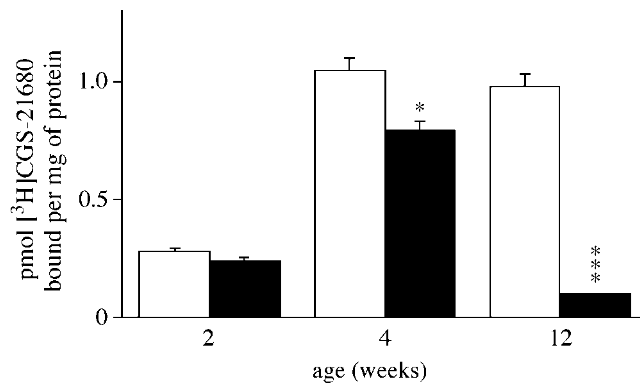

Figure 9. Developmental profile of A2a adenosine receptor binding in control (open bars) and R6/2 transgenic (filled bars) mouse striatum. Numbers are pmol mg^{-1} protein of bound ligand levels (mean ± s.e.m., $n = 6$ animals per group). $^{*}p < 0.01$; $^{***}p < 0.0001$.

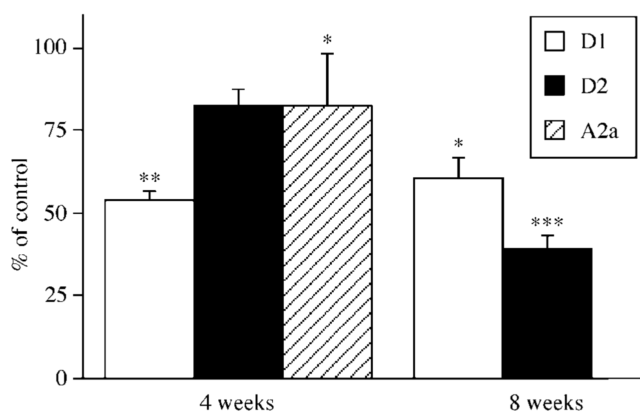

Figure 10. Hybridization *in situ* for A2a adenosine, D_1 dopamine and D_2 dopamine receptor in R6/2 transgenic mouse striatum. Numbers are percentages of control mouse mRNA levels (mean ± s.e.m., $n = 6$ animals). $^{*}p < 0.01$; $^{**}p < 0.001$; $^{***}p < 0.0001$.

had comparable dopamine and adenosine receptor binding levels to those of control mice.

R6/1 transgenic mice develop symptoms between 15 and 21 weeks of age. We performed receptor binding assays on R6/1 transgenic and control mice of various ages (figure 12). D_1 dopamine, D_2 dopamine and A2a adenosine receptor binding were decreased as early as three months of age, before the R6/1 animals become symptomatic.

4. DISCUSSION

Transgenic animals offer a fascinating insight into the pathogenesis of neurodegenerative diseases. Traditional post-mortem human studies are confounded by neuronal loss. Within weeks to months, transgenic mice can recapitulate degenerative processes that usually occur over the course of years. If transgenic animals faithfully recreate the phenotype of a human neurodegenerative disease, such animals would provide an excellent model for testing therapeutic interventions. However, if therapeutic interventions are to succeed, it is crucial to distinguish those phenotypic changes that cause neuronal damage from those that are merely epiphenomena of neuronal damage.

R6/1, R6/2 and R6/5 mice develop an abnormal neurological phenotype. Several features of these transgenic mice are reminiscent of the HD, namely abnormal movements and loss of body weight. Alteration of neurotransmitter receptors is another feature of HD that seems to be recreated in transgenic animals. This argues further for the validity of the transgenic animals as models for HD, and it also provides the opportunity to investigate the role of neurotransmitter receptor alterations in the pathogenesis of HD.

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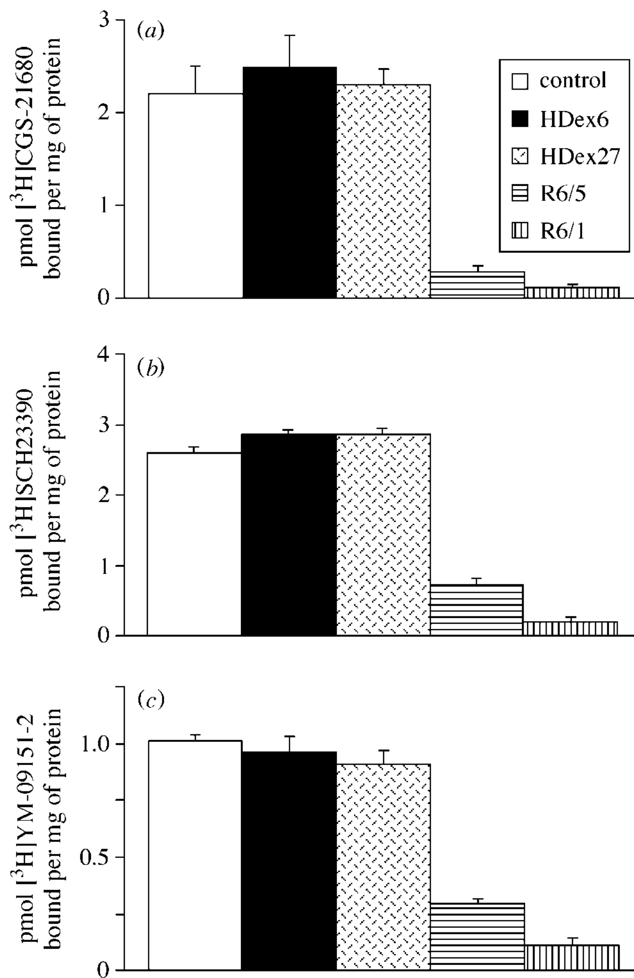

Figure 11. (*a*) A2a adenosine, (*b*) D_1 dopamine and (*c*) D_2 dopamine receptor binding in different strains of transgenic mice containing a portion of the human HD gene. HDex6 and HDex27 are transgenic mouse strains containing a human gene that has 18 repeats (within the 'normal' human range), whereas R6/5 and R6/1 lines have repeat numbers in excess of 100. Numbers represent pmol of ligand bound per mg of protein (mean ± s.e.m, $n = 4$–6 animals per group).

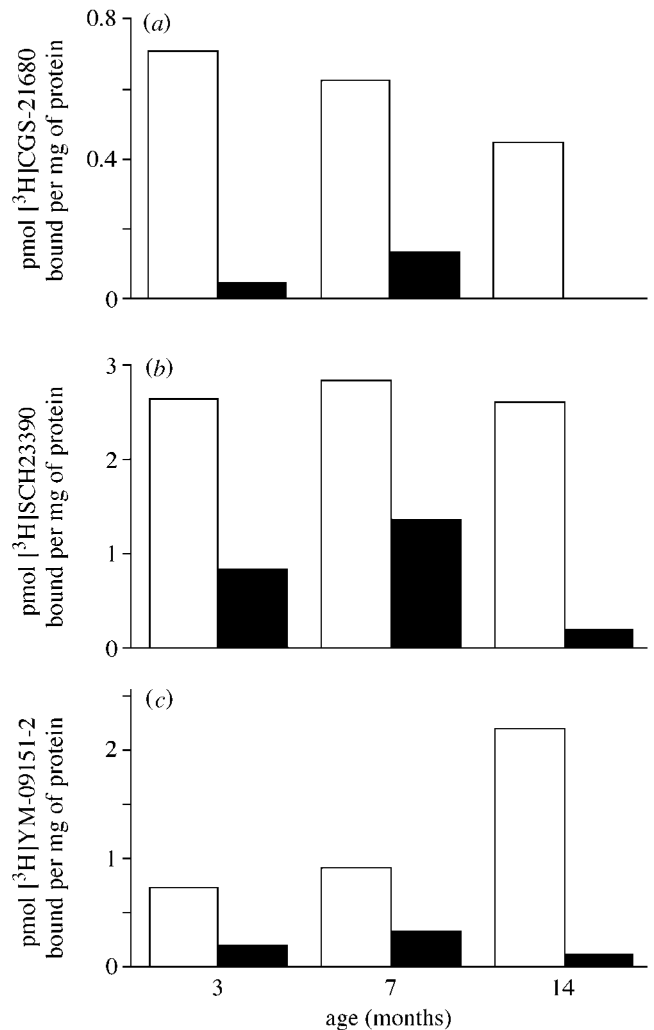

Figure 12. Developmental profile of (*a*) A2a adenosine, (*b*) D_1 dopamine and (*c*) D_2 dopamine receptor binding in control (open bars) and R6/1 transgenic (filled bars) mouse striatum. R6/1 mice develop abnormal symptoms at 15–21 weeks of age.

Receptors that are known to be altered in HD (glutamate, dopamine, muscarinic acetylcholine and adenosine receptors) are also decreased in the brains of 12-week-old R6/2 mice. Importantly, at this age, there is no evidence of neuronal loss (Mangiarini *et al.* 1996). Therefore, the observed decreases in neurotransmitter receptors are unlikely to be the result of simply the loss of striatal neurons. Another possibility is that a certain subpopulation of striatal neurons is lost. It is extremely unlikely that these changes are due to non-specific dysfunction of a specific striatal cell type. The predominant neurons in the striatum (more than 90%) are the GABA-containing medium spiny neurons. Our current findings indicate a decrease in the mGluR1, mGluR3, dopamine and muscarinic cholinergic receptors, without a concomitant decrease in the AMPA, NMDA-NR1, mGluR5 or $GABA_A$ receptors that are contained within the same medium spiny striatal neurons. The pattern of receptor decreases cannot be explained solely by damage to a particular subset of striatal neurons. The present data argue that the observed alterations occur at the level of specific receptors within a given cell type, rather than in all genes of a subpopulation of neurons.

Although neurotransmitter receptors are known to be affected early in the course of HD, it is still unclear whether receptor loss is a primary or a secondary phenomenon. Here again, transgenic animals can yield information. The developmental studies indicate that neurotransmitter receptor decreases occur before the onset of clinical symptoms and neuronal death. Indeed, an altered complement of striatal neurotransmitters could render the striatum more susceptible to excitotoxic insult and resultant neuronal degeneration (DiFiglia 1990; Albin & Greenamyre 1992). Similarly, altered striatal neurotransmitters are likely to contribute to the abnormal movements observed in these transgenic mice.

The hybridization experiments *in situ* indicate that the neurotransmitter receptor decreases are themselves preceded by selective decreases in corresponding mRNA species. Decreased mRNA levels suggest that there is either selective degradation of mRNA species, or, more probably, specific transcriptional down-regulation of particular genes. The current data suggest that transcriptional

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dysfunction might be an important feature in transgenic mouse models of HD. One possible explanation would involve NII, whose presence in the nuclei of transgenic mouse brain neurons could conceivably alter the transcriptional programme of these neurons.

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