

Inhibition of tryptophan hydroxylase activity and decreased 5-HT_{1A} receptor binding in a mouse model of Huntington's disease

George J. Yohrling IV,* George C.-T. Jiang,† Molly M. DeJohn,* Daniel J. Robertson,‡
Kent E. Vrana‡ and Jang-Ho J. Cha*

*Department of Neurology, Massachusetts General Hospital, Charlestown, Massachusetts, USA

†Molecular Genetics Program, ‡Department of Physiology and Pharmacology, Wake Forest University School of Medicine, Winston-Salem, North Carolina, USA

Abstract

The pathogenic mechanisms of the mutant huntingtin protein that cause Huntington's disease (HD) are unknown. Previous studies have reported significant decreases in the levels of serotonin (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the brains of the R6/2 transgenic mouse model of HD. In an attempt to elucidate the cause of these neurochemical perturbations in HD, the protein levels and enzymatic activity of tryptophan hydroxylase (TPH), the rate-limiting enzyme in 5-HT biosynthesis, were determined. Enzyme activity was measured in brainstem homogenates from 4-, 8-, and 12-week-old R6/2 mice and compared with aged-matched wild-type control mice. We observed a 62% decrease in brainstem TPH activity ($p = 0.009$) in 4-week-old R6/2 mice, well before the onset of behavioral symptoms. In addition, significant decreases in TPH activity were also observed at 8 and 12 weeks of age (61%, $p = 0.02$ and 86%, $p = 0.005$, respectively). In the 12-week-old-mice, no change in immunoreactive TPH was observed. *In vitro* binding showed

that TPH does not bind to exon 1 of huntingtin in a polyglutamine-dependent manner. Specifically, glutathione-S-transferase huntingtin exon 1 proteins with 20, 32 or 53 polyglutamines did not interact with radiolabeled tryptophan hydroxylase. Therefore, the inhibition of TPH activity does not appear to result from a direct huntingtin/TPH interaction. Receptor binding analyses for the 5-HT_{1A} receptor in 12-week-old R6/2 mice revealed significant reductions in 8-OH-[³H]DPAT binding in several hippocampal and cortical regions. These results demonstrate that the serotonergic system in the R6/2 mice is severely disrupted in both pre-symptomatic and symptomatic mice. The presymptomatic inhibition of TPH activity in the R6/2 mice may help explain the functional consequences of HD and provide insights into new targets for pharmacotherapy.

Keywords: brainstem, GST pull-down, Huntington's disease, receptor binding, serotonin, tryptophan hydroxylase.

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disease that is caused by a mutation in the gene encoding the huntingtin (Htt) protein (Huntington's Disease Collaborative Research Group 1993). Huntingtin is ubiquitously expressed throughout the central nervous system and its exact function remains unknown. The genetic basis of HD is an expansion of CAG nucleotide repeats in the 5' coding region of the *HD* gene that translates to a polyglutamine expansion in the amino terminus of Htt. When the mutation results in a polyglutamine stretch of 36 or more, a regionally specific degeneration of the striatum occurs. However, pathological and functional abnormalities are present in other brain regions such as the cerebellum,

cerebral cortex and hippocampus (Vonsattel *et al.* 1985). The polyglutamine expansion also causes aberrant protein–protein interactions with a number of important proteins such as nuclear corepressor, p53, CREB binding protein (CBP) and

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Address correspondence and reprint requests to Dr George J. Yohrling IV, Neurology Research, Massachusetts General Hospital, 114 16th Street, B114-2250, Charlestown, MA 02129–4404, USA.

E-mail: yohrling@helix.mgh.harvard.edu

Abbreviations used: CBP, CREB binding protein; GST, glutathione-S-transferase; HD, Huntington's disease; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; Htt, huntingtin; TPH, tryptophan hydroxylase.

PSD95 (Boutell *et al.* 1999; Steffan *et al.* 2000; Nucifora *et al.* 2001; Sun *et al.* 2001). Polyglutamine motifs are not present in all these bound proteins and therefore are not required for Htt binding. It is likely there are other heretofore unidentified Htt-interacting proteins that play a role in the etiology of HD.

Since the identification of the human *HD* gene in 1993, extensive progress has been made in understanding the disease. However, the exact function of the wild-type huntingtin protein is not known, nor are the molecular mechanisms leading to the devastating pathogenesis observed in HD. While neurochemical alterations in human HD cases have long garnered attention from researchers, the confounding issue of cell loss hampers interpretation of neurochemical alterations in post-mortem HD samples. For this reason, transgenic mouse HD models are useful in shedding light on the molecular pathogenesis of HD. In 1996, the first genetic mouse models of HD were introduced (Mangiarini *et al.* 1996). The R6/2 mouse expresses exon 1 of the human huntingtin gene and 262 base pairs of intron 1, under the control of the human HD promoter. These mice exhibit a progressive disease phenotype that includes motor, cognitive, and neuronal disturbances similar to those observed in human HD (Mangiarini *et al.* 1996; Carter *et al.* 1999; Lione *et al.* 1999). However, despite these symptomatic similarities to human HD, R6/2 mice demonstrate little neuronal death.

Initial neurochemical characterization of the R6/2 mice revealed a loss of glutamate and dopamine receptors, both of which are pathologic hallmarks of HD (Cha *et al.* 1998). Decreases in D1 receptor mRNA expression were observed at just 4 weeks of age, well before the onset of both behavioral and motor symptomatology. Similar changes are also present in the R6/1 and R6/5 transgenic HD mice lines (Cha *et al.* 1999). Since then, other gene mRNA changes, such as tenascin-C, dopamine- and cAMP-regulated phosphoprotein (DARPP-32), and cannabinoid receptors (CB1) have been found to be decreased as early as 4 weeks of age (Bibb *et al.* 2000; Denovan-Wright and Robertson 2000; Kusakabe *et al.* 2001). Gene expression profiling of these mice has also revealed that distinct mRNA changes related to calcium homeostasis, signal transduction and neurotransmitter systems occur in the R6/2 mice (Luthi-Carter *et al.* 2000).

Recently, Reynolds and colleagues have reported deficits in several important neurotransmitters in the R6/2 mice (Reynolds *et al.* 1999). They used high-pressure liquid chromatography (HPLC) to measure the levels of 5-hydroxytryptamine (5-HT) and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA). Significant decreases in 5-HT and 5-HIAA were observed in the striatum, hippocampus and brainstem of 12 week old R6/2 mice. In addition, decreases in 5-HIAA were also recorded in the striatum of both 4- and 8-week-old mice. However, these data do not explain why these important serotonergic markers are decreased.

Tryptophan hydroxylase (TPH; EC 1.14.16.4) is a member of the aromatic amino acid hydroxylase superfamily, and serves as the rate-limiting enzyme in the biosynthesis of the neurotransmitter serotonin. Serotonin (5-HT) is an important monoamine that has been implicated in a wide variety of central nervous system functions, including depression, aggression, pain, memory, sleep and temperature control (Kandasamy and Williams 1984; Cornwell-Jones *et al.* 1989; Mockus and Vrana 1998). TPH is a very unstable enzyme with an *in vitro* half-life of 11 min (Mockus *et al.* 1997). In addition, TPH is readily susceptible to enzymatic inhibition by reactive oxygen species, similar to that which has been proposed as a potential mechanism of pathogenesis in HD (Young 1993; Cash 1998). TPH-containing cell bodies reside predominantly in the raphe nuclei of the brainstem. However, due to the extremely low TPH mRNA levels in the brain, northern blot analyses for alterations in TPH message are difficult to interpret. Aberrations in 5-HT synthesis and regulation have also been linked to a diverse group of other neurological and psychiatric disorders such as depression, Parkinson's disease, Tourette's syndrome, multiple sclerosis, and obsessive-compulsive disorder (Tabaddor *et al.* 1978; Volicer *et al.* 1985a,b; Schauenburg and Dressler 1992; Delgado and Moreno 1998). Therefore, a potential target in the etiology of many of these diseases may be TPH.

Decreased levels of 5-HT and 5-HIAA indicate early dysfunction of the serotonergic system. Therefore, we investigated levels of TPH protein and TPH enzymatic activity in R6/2 mice and wild-type littermate control mice at both presymptomatic (4 weeks) and symptomatic (8 and 12 weeks) ages. In addition, we performed serotonin receptor (5-HT_{1A}) binding assays on 12-week-old R6/2 mice. This would allow us to determine if the decreased brain 5-HT levels engendered a postsynaptic compensation, or up-regulation, of 5-HT receptors in the HD mouse model.

Materials and methods

Animals

Transgenic mice that contain exon 1 of the human huntingtin gene (R6/2) and age-matched littermate control mice were killed by decapitation at 4, 8, and 12 weeks of age. The brains were removed and the brainstem was dissected, weighed, and stored at -80°C until TPH activity and protein analyses could be performed. Brains were kindly provided by Dr Gillian Bates (GKT School of Medicine, London, UK). A hemisphere of each 12-week-old mouse brain was parasagittally cut on a cryostat into 12 μm slices onto glass microscope slides (SuperFrost Slides, Fisher Scientific, Pittsburgh, PA, USA). The sections were stored at -80°C until receptor binding analyses could be performed.

Materials

All materials were obtained from Sigma (St Louis, MO, USA) with the following exceptions; L-[5-³H] tryptophan was from DuPont-NEN

Research Products (Boston, MA, USA); and activated charcoal (Darco G-60) was from Fisher Scientific. Reinforced nitrocellulose (Duralose-UV) and was purchased from Stratagene (La Jolla, CA, USA). Prestained protein ladder was purchased from Life Technologies (Gaithersburg, MD, USA). Chemiluminescent reagents (Renaissance) were purchased from NEN Research Products. The mouse anti-TPH monoclonal antibody (WH3) was obtained from Research Biochemicals Inc. (Natick, MA, USA). The sheep anti-mouse IgG HRP-coupled secondary antibody, 8-OH-[³H]DPAT and tritium-sensitive film (Hyperfilm) were purchased from Amersham Life Sciences (Arlington Heights, IL, USA). The GST-Htt clones (CAG20Q, CAG32Q and CAG53Q) were a generous gift from Dr Erich Wanker (Max-Planck Institute, Germany).

TPH activity assay

Brainstems were resuspended in 10 μ L of HEPES-based buffer with protease inhibitors (pH 7.0) per mg of tissue. Tissue samples were sonicated three times at 30% power for 10 s each with a Vibra Cell Sonicator (Sonic and Materials, Danbury, CT, USA). TPH activity was determined on 25 μ L aliquots for each animal (in duplicate) using a radioenzymatic ³H₂O release assay as previously described (Beevers *et al.* 1983; Vrana *et al.* 1993). The only significant variation to this method was that a final concentration of 50 μ M BH₄ was utilized to minimize substrate inhibition of TPH. Activity values derived from each assay were normalized to total protein present in the homogenates, as determined by Bradford protein assay, and were expressed as nmol/h/mg.

TPH protein analysis

Denaturing polyacrylamide gel electrophoresis (10% acrylamide, 0.27% bis-acrylamide) was performed on equal total protein aliquots (25 μ g) of brainstem homogenates for six 12-week-old control mice and six 12-week-old R6/2 mice. The proteins were resolved at 120 V for 2 h and then transferred to Duralose-UV (Stratagene) membrane using a semidry electroblot apparatus set at 400 mA for 1.5 h (Owl Scientific, Cambridge, MA, USA). BenchmarkTM prestained protein ladder was resolved with the samples (Life Technologies). After transfer, TPH proteins were detected by probing with a 1 : 1000 dilution of affinity-purified mouse anti-TPH monoclonal antibody followed by a 1 : 1500 dilution (1.5 mg/mL) of sheep anti-mouse IgG coupled with HRP. The TPH mouse monoclonal antibody recognizes an epitope between residues 103 and 109 (Haycock *et al.* 2002). The incubation and wash conditions for western analyses are outlined in Mockus *et al.* (1997). Immune complexes were visualized using enhanced chemiluminescence (NEN Research Products) and exposed to X-ray film (Kodak Bio-Max MR2). Densitometric analysis of the film was performed to determine if there were any changes in TPH immunoreactivity that would correspond to the decreases in TPH activity. The TPH blot was stripped, blocked with 5% dry milk in TBS-T, and re-probed with a mouse anti-actin monoclonal antibody (Chemicon, Temecula, CA, USA) at a 1 : 1000 dilution to control for protein loading.

GST protein production and pull-down assays

Glutathione-S-transferase (GST) huntingtin exon 1 fusion proteins that were described previously, were expressed in BL21 (DE3) *Escherichia coli* with 0.1 mM isopropylthio- β -D-galactosidase at 30°C for 2.5 h (Scherzinger *et al.* 1997). Following protein

induction, the bacteria were lysed with lysozyme and brief sonication and the soluble proteins were recovered. Each GST clone was then purified with Glutathione Sepharose 4B beads (Amersham Pharmacia Biotech AB, Uppsala, Sweden). Verification of protein synthesis was performed with SDS-PAGE and Coomassie Blue staining. Equal amounts of GST-CAG20Q, GST-CAG32Q and GST-CAG53Q were incubated with 5 μ L of ³⁵S-methionine-labeled *in vitro* translated pET21c-TPH construct for 1.5 h at room temperature (22°C). The GST proteins were washed 3 times with 500 μ L of binding buffer (20 mM Tris pH 7.5, 150 mM NaCl, 0.1 mM EDTA, 1 mM dithiothreitol, 0.3% IEGPAL, and 20% glycerol). The proteins were then eluted in 30 μ L of Laemmli buffer, boiled for 5 min, and run on a 10% Bis-Tris acrylamide gel (Invitrogen, Carlsbad, CA, USA). The proteins were resolved at 120 V for 2 h and stained with Coomassie Blue solution for 30 min. The gel was then destained overnight with 10% acetic acid, dried under vacuum and heat (50°C) for 2 h and exposed to Bio-Max autoradiographic film (Kodak).

Serotonin receptor (5-HT_{1A}) binding

5-HT_{1A} (5-hydroxytryptamine) receptor binding was performed according to protocols described by McKittrick *et al.* (1995) and Verge *et al.* (1986). Slides were removed from freezer to thaw for 1 h before a 30-min prewash in room temperature buffer (50 mM Tris-HCl pH 7.4, 5 mM CaCl₂, 180 mM NaCl, 1.2 mM MgCl₂). Slides were then incubated in 1.5 nM [³H]8-hydroxy-2-dipropylaminotetralin (8-OH-[³H]DPAT, specific activity 234 Ci/mmol, Amersham Pharmacia Biotech (Arlington Heights, IL, USA) and 10 μ M pargyline (Sigma) at room temperature for 1 h. 5-HT-displaceable 8-OH-[³H]DPAT binding was defined with duplicate sections incubated with 8-OH-[³H]DPAT in the presence of 1.0 μ M 5-HT (Sigma). Slides were rinsed in two washes, each for 5 min, in 4°C buffer followed by a quick rinse in 4°C ddH₂O. Slides were dried under a cool air stream and then apposed to tritium-sensitive film (Hyperfilm ³H, Amersham Pharmacia Biotech, Arlington Heights, IL, USA) with calibrated radioactive standards for 3 weeks. Film was developed and analyzed using a computer image analysis system (M1, Imaging Research, St Catharines, Ont., Canada). The image density that corresponds to the binding of [³H] ligand was converted from optical density to pmol/mg protein with calibrated radioactive standards. Non-specific binding was subtracted.

Statistical analyses

The TPH activity, protein, and 5-HT_{1A} receptor binding, data were analyzed using one-way analysis of variance (ANOVA), followed by unpaired Student's *t*-tests. Statistical significance was associated with values of *p* < 0.05.

Results

Western analysis was performed on 25 μ g of total brainstem protein from six 12-week-old wild-type littermate control mice and six 12-week-old R6/2 mice. TPH immunoreactivity was observed at the predicted molecular weight of 51 kDa (Fig. 1a, upper blot). In addition, actin immunoreactivity was measured on the same blot to correct for protein loading differences (Fig. 1a, lower blot). The TPH and actin bands were subjected to densitometric analyses at several different

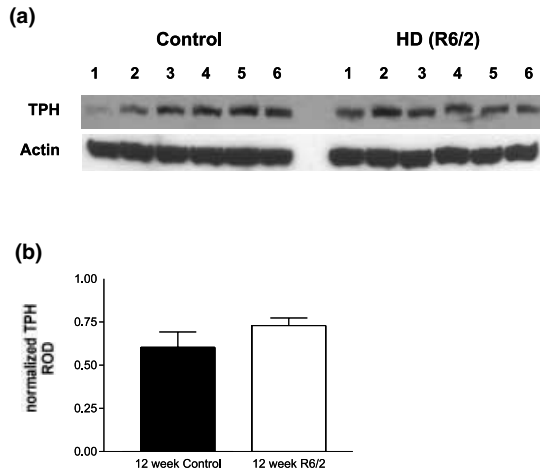


Fig. 1 (a) Western analysis of TPH immunoreactive protein. 25 μ g of total brainstem protein from 12-week-old R6/2 and control mice was visualized with an anti-TPH monoclonal antibody (WH3). TPH was visualized at the predicted molecular weight of 51 kDa. The blot was stripped and re-probed for actin (mouse anti-actin; Chemicon) to normalize protein loading. (b) Densitometric analysis of TPH protein. The blots in Fig. 1a were subjected to densitometric analyses. TPH signal from each animal was normalized to actin signal. No significant changes are observed in TPH immunoreactivity for the R6/2 and control mice ($p = 0.23$).

exposure times to confirm that the serotonergic metabolite level changes that were previously observed were not merely due to a loss of immunoreactive protein (Fig. 1b). There was no change in TPH protein ($p = 0.23$) in the 12-week-old mice.

We observed significant decreases in TPH activity in all time points tested (Fig. 2). At the presymptomatic time point of 4 weeks, TPH activity was decreased by 62% in the R6/2 mice (Figs 2a; $p = 0.009$). TPH activity after 8 weeks was similarly decreased by 61% (Figs 2b; $p = 0.02$). At this age, the animals typically have numerous behavioral abnormalities including a resting tremor and cognitive dysfunction (Carter *et al.* 1999; Lione *et al.* 1999). We saw the most pronounced decrease in TPH activity in the 12-week-old R6/2 mice (Figs 2c; $p = 0.005$). Activity was decreased by 86% in the 12-week-old R6/2 mice compared to their age-matched controls.

Given that expansion of the polyglutamine stretch in Htt often produces aberrant protein-protein interactions, an *in vitro* binding assay was used to investigate if a TPH-Htt interaction is occurring in a polyglutamine-dependent fashion. GST pull-down assays showed that huntingtin exon 1 GST constructs containing 20, 32 or 53 polyglutamines do not interact with a radiolabeled full-length TPH construct (Fig. 3). None of the GST exon 1 constructs tested demonstrated an increased interaction with TPH when compared to the GST only sample. These results were replicated three times to verify the lack of interaction. The proteins on all gels

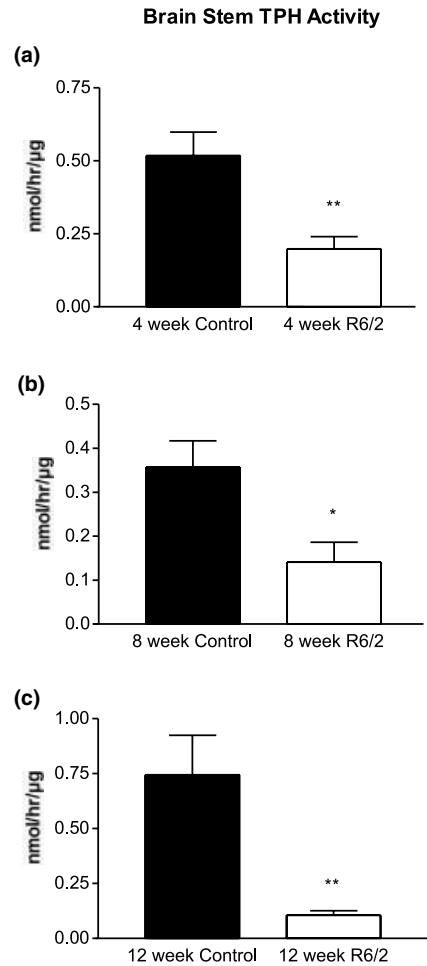


Fig. 2 Brainstem TPH activity in 4, 8, and 12-week-old R6/2 and control mice. (a) Brainstem TPH activity in 4-week-old R6/2 mice. Mean TPH activity for the 4-week controls was 0.518 nmol/h μ g⁻¹. TPH activity for the 4-week R6/2 mice was 0.199 nmol/h μ g⁻¹, a 62% reduction ($p = 0.009$). (b) Brainstem TPH activity in 8-week R6/2 mice. Mean TPH activity for the 8-week controls was 0.357 nmol/h μ g⁻¹. TPH activity for the 8-week R6/2 mice was 0.141 nmol/h μ g⁻¹, a 61% reduction ($p = 0.02$). (c) Brainstem TPH activity in 12-week R6/2 mice. Mean TPH activity for the 12-week controls was 0.745 nmol/h μ g⁻¹. TPH activity for the 12-week R6/2 mice was 0.106 nmol/h μ g⁻¹, which represents an 86% reduction ($p = 0.005$). TPH activity is represented as nmol/h/mg of total protein. * $p < 0.05$, ** $p < 0.01$.

were stained with Coomassie Blue to verify that equal amounts of each GST huntingtin exon 1 protein were used in the pull-down reactions (data not shown). These GST huntingtin exon 1 constructs have previously been utilized to precipitate other radiolabeled proteins of interest, such as p53 (Steffan *et al.* 2000).

We used 8-OH-[³H]DPAT, a selective 5-HT_{1A} agonist, as a measure of 5-HT_{1A} receptors in the brains of 12-week-old R6/2 and wild-type littermate control mice (Fig. 4). This

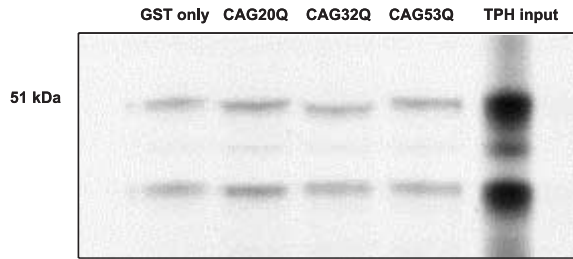


Fig. 3 GST pull-down assay. GST huntingtin exon 1 constructs with 20, 32, and 53 polyglutamines (CAG) were unable to pull-down a ^{35}S -methionine labeled TPH cDNA (Vrana *et al.* 1994). There is no significant difference in Htt binding between the GST only sample (negative control) and the exon 1 Htt constructs. The input lane (1 μL of the *in vitro* translated product) demonstrates where TPH should be expected to migrate (51 kDa). The lower molecular weight band is likely a truncated TPH isoform that has been *in vitro* translated at a downstream ATG initiation codon. These GST-fusion constructs have previously been shown to bind other radiolabeled proteins (Steffan *et al.* 2000).

would permit us to determine if decreased brain 5-HT levels cause a postsynaptic compensation of 5-HT receptors in the R6/2 mice. Significant reductions in 8-OH- ^3H DPAT binding were observed in the hippocampus (CA1 and CA3), as well as the entorhinal cortex, and inner and outer frontal cortices of the R6/2 mice (Fig. 4d).

Discussion

Huntington's disease is a devastating neurodegenerative disease with no known cure. While the gene that causes HD has been identified, the exact mechanisms that cause the disease remain a mystery. It appears that the neuropathology of HD may manifest itself through numerous mechanisms such as transcriptional dysregulation, excitotoxicity, proteolysis and mitochondrial dysfunction (DiFiglia 1990; Young 1993; Petersén *et al.* 1999; Ross *et al.* 1999; Cha 2000). In the present studies, we found no difference in TPH protein levels at 12 weeks of age in spite of significantly decreased TPH activity at 4, 8 and 12 weeks of age. The inhibition of TPH however, does not appear to be due to a polyglutamine-dependent protein-protein interaction. Receptor binding analyses revealed significant reductions in 8-OH- ^3H DPAT binding at the 5-HT $_{1A}$ receptor throughout the hippocampus and cortex of the 12-week-old R6/2 mice. These data provide strong evidence that both large presymptomatic and symptomatic serotonergic abnormalities occur in the R6/2 mouse model of HD.

It is possible that TPH is inhibited in the R6/2 animals because of the increased oxidative stress that has been postulated in these mice (Tabrizi *et al.* 2000). Specifically, Tabrizi and colleagues found significant reductions in aconitase and mitochondrial complex IV activities in the striatum of 12-week-old R6/2 mice. In addition, increased

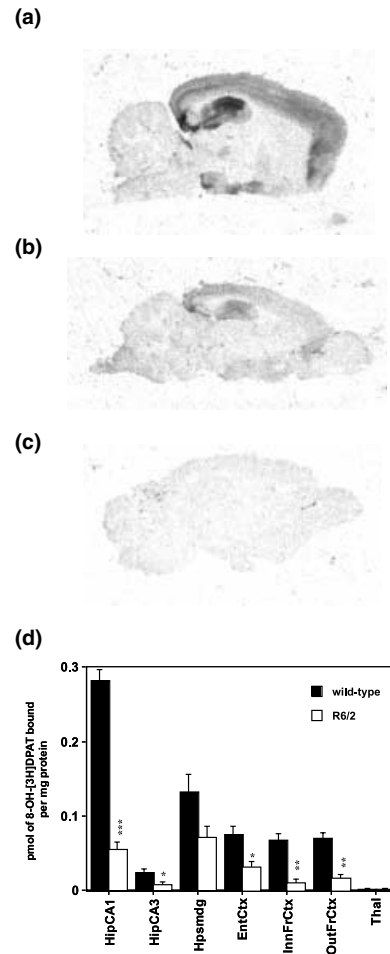


Fig. 4 Serotonin receptor binding. (a) Representative 8-OH- ^3H DPAT binding in a 12-week-old wild-type littermate control mouse. (b) Representative 8-OH- ^3H DPAT binding in a 12-week-old R6/2 mouse. (c) Background binding in a 12-week-old wild-type littermate control mouse. (d) The mean cell densities for 8-OH- ^3H DPAT binding \pm SEM ($n = 5$) in the wild-type (black bars) and R6/2 (white bars) mice are shown as pmol of 8-OH- ^3H DPAT bound per mg of total protein. 5-HT $_{1A}$ binding was measured in the CA1 region of the hippocampus (HipCA1), CA3 region of the hippocampus (HipCA3), striatum moleculare of the dentate gyrus (Hpsmdg), entorhinal cortex (EntCtx), inner frontal cortex (InnFrCtx), outer frontal cortex (OutFrCtx), and thalamus (Thal). Binding to 5-HT $_{1A}$ receptors was significantly decreased in all regions tested except the thalamus and striatum moleculare of the dentate gyrus. No 5-HT $_{1A}$ binding was observed in the striatum or cerebellum. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

immunostaining for inducible nitric oxide synthase (iNOS) and nitrotyrosine were seen in the R6/2 mice. Such elevations can be directly correlated to increased nitric oxide and subsequent free radical formation, both of which can inhibit TPH and other members of the aromatic amino acid hydroxylase family (Fleckenstein *et al.* 1997; Cash 1998; Blanchard-Fillion *et al.* 2001).

Tryptophan, the amino acid substrate for TPH, also forms nicotinamide adenine dinucleotide (NAD⁺) and a number of reactive intermediates called kynurenines, through what is commonly called the kynurenine pathway. Recently, early stage impairment of the kynurenine pathway has been observed in the striatum of human grade 1 HD patients, as well as in transgenic mice expressing the full-length huntingtin gene (Pearson *et al.* 1995; Guidetti *et al.* 2000). Specifically, Guidetti and colleagues found large increases in 3-hydroxykynurenine (3HK), a free-radical generator, in both the human and transgenic mouse brain. If 3HK is truly a common pathologic marker of HD, it is possible that free radicals may play a role in the presymptomatic inhibition of TPH activity in the R6/2 mice.

Recently, several studies have reported early gene, receptor and protein changes in HD mouse models (Cha *et al.* 1998; Cha *et al.* 1999; Denovan-Wright and Robertson 2000; Deckel *et al.* 2001; Kusakabe *et al.* 2001; Morton and Edwardson 2001). In addition, the polyglutamine expansion in the Htt protein has been shown to bind and sequester important transcription factors which leads to altered transcriptional activation (Steffan *et al.* 2000; Nucifora *et al.* 2001). Deficiencies in cAMP regulated genes have also been reported in the R6/2 mice (Bibb *et al.* 2000; Luthi-Carter *et al.* 2000). Although TPH mRNA levels are difficult to measure, it is possible that the transcriptional aberrations that have been described in several HD models, may play a role in the TPH inactivation we observed in these studies. For example, the human TPH promoter contains a cAMP-responsive element that, in the presence of cAMP, increases TPH gene expression (Boularand *et al.* 1995). Recently, researchers found that rats injected with sertraline, a potent serotonin-selective reuptake inhibitor (SSRI), have elevated levels of TPH activity, protein, and mRNA (Kim *et al.* 2002). This up-regulation is attenuated with the PKA inhibitor, H-89, suggesting that the cAMP/PKA pathway is involved in the serotonergic changes in the treated rats. Therefore, it is possible that cAMP deficiencies in the R6/2 mice may disrupt TPH gene expression.

Given the decrease in 5-HT and 5-HIAA, the finding that 5-HT_{1A} binding was decreased throughout the hippocampus and cortex was unexpected. We predicted that 5-HT_{1A} binding would increase in response to the lowered brain serotonin and TPH activities. Up-regulation of the 5-HT_{1A} receptor subtype has previously been demonstrated to compensate for the serotonergic dysfunction in other animal models of neurodegenerative diseases, such as Parkinson's disease (Frechilla *et al.* 2001). However, in a study investigating binding of another serotonin receptor (5-HT_{1B}) in human HD cases, it was shown that 5-HT_{1B} binding is also decreased (Castro *et al.* 1998). It is possible that the decreases in 5-HT receptor binding in HD may be a more widespread phenomenon. While receptor number was not measured in the present study, these data do suggest that the polyglutamine

expansion in the R6/2 mice does not cause an up-regulation of this aspect of the serotonergic receptor system.

Our finding that TPH activity is inhibited in the brainstem of the R6/2 mice complements the previous observation that 5-HT and 5-HIAA levels are also decreased in the brainstem of the R6/2 mice (Reynolds *et al.* 1999). However, these findings are in contrast to those that have been observed in human post-mortem HD brains. Specifically, several groups have measured 5-HT and 5-HIAA levels in both the blood and brain of HD patients (Belendiuk *et al.* 1980; Kish *et al.* 1987; Reynolds and Pearson 1987). In all cases, 5-HT and 5-HIAA were increased in HD striatum, but remained relatively unchanged in other brain regions such as the hippocampus and substantia nigra (Kish *et al.* 1987; Reynolds and Pearson 1987). The most obvious reason for these differences is that the R6/2 mouse does not perfectly represent the human HD condition. Another possibility, and a confounding problem with the use of post-mortem HD tissue, is the issue of cell loss. While 5-HT levels may appear to be increased, the striatal atrophy that occurs in HD may account for these findings. To date, it remains unknown if TPH activity is inhibited in human HD brainstem. Such studies should be performed to determine if the R6/2 is the appropriate model for the serotonergic alterations that are seen in HD.

The finding that TPH activity is so significantly reduced at a presymptomatic time point in the R6/2 mice is a potentially important finding. The inhibition of this critical enzyme may help explain the severe depression, cognitive and motor abnormalities that occur in HD (Harper 1996). Depression often precedes the onset of motor abnormalities in HD. It may be possible to administer antioxidants or other pharmacotherapies, such as SSRIs to prevent the inhibition of TPH and bolster brain serotonin levels. Recently, Hickey and colleagues have administered L-DOPA to symptomatic R6/2 mice and were able to observe short-term improvements in both motor activity and rearing behavior (Hickey *et al.* 2002). It is thought that L-DOPA therapy is acting to reverse the decreases in brain dopamine that have also been documented to occur in the R6/2 mice (Reynolds *et al.* 1999). It will be interesting to see if a similar therapy for serotonin/TPH would have a beneficial outcome in the HD pathology of these mice. In addition, it remains to be seen if TPH activity and 5-HT binding are also reduced in other polyglutamine diseases.

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