Changes in Expression of N-Methyl-D-Aspartate Receptor Subunits Occur Early in the R6/2 Mouse Model of Huntington’s Disease

Noore J. Ali    Michael S. Levine

Mental Retardation Research Center, The David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, Calif., USA

Key Words
Huntington’s disease · R6/2 mouse model · N-methyl-D-aspartate receptors · Receptor subunit RNA · Single-cell RT-PCR

Abstract
A leading hypothesis of the cause of neuronal death in Huntington’s disease (HD) is excitotoxicity, in which subpopulations of striatal neurons are hypersensitive to glutamate release due to changes in postsynaptic N-methyl-D-aspartate receptors (NMDARs). In the present study we used RT-PCR methods on single cells and tissue to compare the expression of NMDAR subunits, NR1, NR2A and NR2B, in the striatum of R6/2 transgenic mice with their wild-type (WT) littermates at three different age groups corresponding to different symptomatic milestones (19–25 days showing no overt evidence of abnormal behavior, 38–45 days at the onset of the overt phenotype and 78–90 days displaying the full behavioral phenotype). Single-cell RT-PCR studies also examined neurons for the expression of substance P and enkephalin to define different subpopulations of medium-sized projection neurons of the striatum. The results showed a significant decrease in the percentage of cells expressing NR2A at all ages examined. The decrease in expression was not associated with any significant change in expression of NR1 or NR2B. Cells that did not express NR2A contained both enkephalin and substance P, but proportionately more cells containing enkephalin displayed decreases in NR2A. Semi-quantitative RT-PCR studies on striatal tissue in the oldest age group confirmed the significant decrease in NR2A and also showed a decrease in NR2B. These results support the hypothesis that changes in the composition of postsynaptic NMDARs occur in the R6/2 model of HD and this effect occurs early in the expression of the phenotype.

Introduction
Huntington’s disease (HD) is an inherited autosomal dominant neurodegenerative disorder characterized by progressive motor, psychiatric and cognitive symptoms [Martin and Gusella, 1986]. The neuropathology of the disease preferentially targets the GABAergic medium-sized spiny neurons of the striatum; and there is also a less profound loss of neurons in the deep layers of the cerebral cortex [DiFiglia, 1990; Vonsattel et al., 1985]. HD is caused by an expanded CAG repeat coding for polyglutamine in the N-terminal region of a ubiquitously expressed protein called huntingtin. Normal individuals possess repeat lengths of 6–35 glutamines, while repeat...
lengths greater than 36 are sufficient for the HD phenotype. The age of onset of HD is roughly inversely related to the number of CAG repeats [Vonsattel and DiFiglia, 1998]. In addition, in the striatum, the ‘indirect’ pathway neurons expressing enkephalin (ENK) are affected earlier than the substance P (SP) expressing ‘direct’ pathway neurons [Albin et al., 1989; Menalled et al., 2000].

One hypothesis to explain neuronal death in HD involves excitotoxicity due to dysregulation of glutamate function [Beal et al., 1993; DiFiglia, 1990]. Abnormal glutamate function could be induced by changes in postsynaptic N-methyl-D-aspartate receptors (NMDARs) rendering subpopulations of striatal neurons hypersensitive to glutamate release in HD. There also is a dysfunction of glutamate release from the corticostriatal pathway in HD [Cepeda et al., 2003]. Overactivation of NMDARs results in high calcium influx, triggering calcium-dependent enzymes and the creation of free radical oxygen species that can result in cellular dysfunction [Bonfoco et al., 1995]. Intrastriatal injections of NMDAR agonists in primates and rats mimic many of the neuropathological, biochemical, and behavioral aspects of HD [Beal et al., 1993; Bordelon and Chesselet, 1999; Coyle, 1979; DiFiglia, 1990; Schwartz et al., 1984]. Furthermore, electrophysiological studies have shown a increased responsiveness to NMDA in subpopulations of striatal neurons in transgenic mouse models of HD [Cepeda et al., 2001a; Laforet et al., 2001; Levine et al., 1999; Zeron et al., 2002]. NMDARs in postnatal striatum are composed of combinations of at least one NR1 subunit and one or more NR2A and/or NR2B subunits [Behe et al., 1995; Hollmann et al., 1994]. NR2B subunits are evenly distributed throughout the striatum whereas NR2A subunit expression is greater in the dorsal lateral region [Chapman et al., 2003].

In cell cultures, agonist-dependent cell death occurs in cells expressing both NR1/NR2A and NR1/NR2B combinations; however, cell death is greater for the NR1/NR2B combination [Zeron et al., 2001].

In the present study we employed single-cell and semi-quantitative RT-PCR methods to compare the expression of NR1, NR2A and NR2B in the striatum of the R6/2 mouse model of HD compared to wild-type (WT) age-matched littermates. The R6/2 mouse model, which carries exon 1 of the human HD gene containing about 150 CAG repeats, mimics several aspects of HD including progressive motor [Carter et al., 1999] and cognitive deficits [Lione et al., 1999; Murphy et al., 2000] as well as abnormalities in transmitter and receptor expression [Ariano et al., 2005; Bibb et al., 2000; Cha et al., 1998; Luthi-Carter et al., 2000, 2003; Menalled et al., 2000]. Intracellular protein aggregates and nuclear inclusions appear in the R6/2 mouse models before any overt behavioral symptoms [Davies et al., 1997; Sathasivam et al., 1999]. Three age groups, corresponding to increasing symptomatic milestones were used in the single-cell RT-PCR studies. Neurons also were examined for expression of SP and ENK to define different subpopulations of medium-sized neurons and correlate expression of these markers with the NMDAR subunits. Finally, semi-quantitative RT-PCR was used to examine striatal tissue content of NMDA receptor subunits in the oldest age group.

**Methods**

**Animals**

All procedures were carried out in accordance with the National Institutes of Health’s Guide for Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at the University of California at Los Angeles (UCLA). All animals were initially genotyped before weaning using standard PCR protocols and then re-genotyped after being sacrificed for use in the experiments. Experiments were conducted in 3 groups of R6/2 and age-matched littermate controls (WT). Age groups were defined according to the development of the phenotype, a young group (19–25 days) showing no overt evidence of abnormal behaviors, a middle-aged group (38–45 days), corresponding to the onset of the overt phenotype and an older group (78–90 days), displaying the full behavioral phenotype. At each age, 5 WT animals and 5 R6/2 transgenics were used to harvest neurons for single-cell RT-PCR. Approximately 10 neurons were harvested from each animal (19–25 days, 52 cells from each genotype; 38–45 days, 53 cells from each genotype; 78–90 days, 56 cells from each genotype). All of the animals were obtained from the breeding colony of R6/2 mice at UCLA.

**Cell Dissociation**

Mice were anesthetized with halothane and decapitated. Brains were removed, blocked and sliced into 350-μm coronal sections in cold (4°C) sucrose solution (250 mM sucrose, 11 mM glucose, 15 mM HEPES, 1 mM dibasic Na₂HPO₄, 4 mM MgSO₄, 2.5 mM KCl, 100 μM CaCl₂ and 1% phenol red; pH 7.4 and 300 mOsm) on a DSK microslicer (Ted Pella, Redding, Calif., USA). Slices were incubated for at least 1 h at room temperature in bubbling (95% O₂ and 5% CO₂) sodium bicarbonate-buffered Earle’s salt solution containing 1 mM pyruvic acid, 1 mM kynurenic acid, 0.1 mM N-nitroarginine and 5 μM glutathione (pH 7.4, 300 mOsm). Slices were placed in Na-isethionate buffer (140 mM Na-isethionate, 15 mM HEPES, 2 mM KCl, 2 mM MgCl₂, 100 μM CaCl₂ and 1% phenol red; pH 7.4 and 300 mOsm) and the dorsal lateral region of the striatum was removed under a dissecting microscope. The striatal slices were then placed in oxygenated HEPES-buffered Hanks’ balanced salt solution (HBSS, 15 mM HEPES, 1 mM pyruvic acid, 1 mM kynurenic acid, 0.1 mM N-nitroarginine and 5 μM glutathione; pH 7.4 and 300 mOsm) containing 0.5 mg/ml protease (type
XIV) at 37°C for 20 min. The tissue was then removed from the enzymatic solution and rinsed 3 times in Na-isethionate buffer. Fire-polished Pasteur pipettes were used to triturate the tissue in the Na-isethionate buffer. The cell suspension was then placed in 35-mm Petri dishes (Nunc, Naperville, Ill., USA) and mounted on an upright fixed-stage microscope (Zeiss Axioscope, Thornwood, N.Y., USA). The cells were allowed 10 min to settle to the bottom of the plate after which oxygenated background solution (140 mM NaCl, 23 mM glucose, 15 mM HEPES, 2 mM KCl, 2 mM MgCl₂, 1 mM CaCl₂ and 1% phenol; pH 7.4 and 300 mOsm) was perfused continuously onto the cells. Electrodes that were previously baked for 4 h at 200°C were pulled (P-97, Sutter Instrument Co., Novato, Calif., USA) and the tip of the electrode was filled with 5 µl RNase-free H₂O. Individual, healthy (intact cell membrane) medium-sized striatal neurons were collected with the electrodes.

**Single-Cell RT-PCR**

The individual cells were ejected into 0.5-ml thin-walled PCR tubes with 15 µl RT mix containing 1 µl Sensiscript RT (200 U/µl), 2 µl of 10 X RT buffer, 1 µl dNTPs (0.5 mM), 0.5 µl RNasin (28,000 U/µl), 0.6 µl random primers (3 µg/µl), 0.5 µl oligo(dT) (0.5 µg/ml) and 8.4 µl RNase-free H₂O. RNasin, random primers and oligo(dT) were purchased from Invitrogen (Carlsbad, Calif., USA) and all other reagents were from Qiagen (Valencia, Calif., USA) Sensiscript kit. The reaction took place at 37°C for 1 h.

An Eppendorf mastercycler was used to perform all of the PCR reactions. PCR amplification was performed using a Qiagen Hotstart kit. For detection of SP, ENK and β-actin (for internal control), 1 µl of template was added to thin-walled 0.2 ml PCR tubes containing 10 µl mastermix, 7.8 µl H₂O, and 1.2 µl of primer mix (10 µM each of upstream and downstream primer). The thermocycling reaction was carried out at 95°C for 12 min, followed by 45 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min and finally 72°C for 10 min. For detection of NR1, NR2A and NR2B, 10 µl of template was added to thin-walled PCR tubes containing 25 µl of Hotstart mastermix, 12.5 µl H₂O, 0.5 µl NR1, 1 µl NR2A, 1 µl NR2B primer mixes (10 µM each of upstream and downstream primer). The first round thermocycling reaction was carried out at 95°C for 12 min, followed by 30 cycles of 94°C for 45 s, 59°C for 45 s and 72°C for 1 min and then 72°C for 10 min. For the second round amplification reactions, 2 µl of template from the previous reaction was added to separate PCR tubes containing a mix of 10 µl Hotstart mastermix, 6.8 µl H₂O and 1.2 µl of either NR1, NR2A or NR2B primer mix (10 µM each of upstream and downstream primer). The second round reaction was performed at 95°C for 5 min followed by 25 cycles of 94°C for 1 min, 58°C for 1 min and 72°C for 90 s and then 72°C for 10 min. PCR products were separated by gel electrophoresis on a 1.5% trevigel (Gaithersburg, Mich., USA) and visualized by staining with ethidium bromide.

Negative controls were added to each RT-PCR procedure for genomic and extraneous DNA. For genomic DNA control, medium-sized neurons were added to a reaction mixture without RT. To control for extraneous DNA, RT reactions were performed with the RT mix minus cells. Positive controls for the PCR reaction were performed with cDNA generated from striatal tissue.

Primers used for the PCR reactions were as follows:

**ENK**

(sense): 5'-GACAGCAGCAACAACAGGATGA-3', (antisense): 5'-AGGCGCAGACTCAGCAGAAAG-3' (product size 475 bp).

**SP**

(sense): 5'-AGCCTCAGAGTTTCTTGGGA-3', (antisense): 5'-CAGCATGAAAGCAGAACCAG-3' (product size 585 bp).

**NR1**

(sense): 5'-GCTGTACCTGTGGACCGCT-3', (antisense): 5'-GCAGTGTAAGGCAAGGCTATGATC-3' (product size 219 bp).

**NR2A**

(sense): 5'-GCTACGGGCGACGAGAAAG-3', (antisense): 5'-GTGGTTGCTACCTGGCTCACC-3' (product size 257 bp).

**NR2B**

(sense): 5'-TGATGCCTACCTGTGGACGCT-3', (antisense): 5'-CCGGAATCTCGTACTCCTGTT-3' (product size 577 bp).

**Semi-Quantitative RT-PCR**

These experiments were performed using an additional 6 R6/2 and 6 WT mice. Total striatal RNA from 78- to 90-day-old WT and R6/2 mice was isolated using an RNasy kit (Qiagen). A Sensiscript kit (described earlier) was used for RT of the 4 µg total RNA. PCR primers and cycling conditions for each gene (NR1, NR2A, NR2B and β-actin) were as detailed earlier. The concentration of starting cDNA was to be amplified for each subunit was determined by building a standard curve for each gene, plotting the density of the PCR product against the amount of template cDNA. One point in the linear range (when the concentration of the DNA was directly proportional to the density of the PCR product) was chosen to compare the relative expression of the gene of interest to the selected reference gene (β-actin). PCR reactions were performed in triplicate with internal positive (genomic DNA) and negative (no cDNA template) controls. Gel band densities were determined using Scion GelPlot 2 and expressed as a ratio to β-actin for each individual sample.

**Statistics**

Statistical differences were assessed by appropriate ANOVAs followed by t tests for individual groups using appropriate error terms from the ANOVA. Differences between means were considered statistically significant if p < 0.05.

**Results**

**Changes in Expression of NMDAR Subunits**

All of the cells at each age were examined for expression of SP and ENK as well as the presence of NR1, NR2A and NR2B subunits (fig. 1). The percentage of cells expressing a particular gene was averaged for each mouse and then for the total number of WT or R6/2 mice at each age to provide a mean and a standard error. First, the data were examined for changes in mean percentage of neurons expressing SP and ENK between WT and R6/2 transgenic mice using a three-way ANOVA (fig. 2). There were no significant differences in the mean percentages of neurons expressing SP and ENK across the age groups or between genotypes. The data were then examined for
expression of the NMDAR subunits using a similar three-way ANOVA. There was a significant interaction between genotype and subunit ($F = 8.64$, d.f. = 2,22, $p = 0.0017$), indicating that the NMDAR subunits were differentially distributed between neurons from WT and R6/2 transgenic mice. There was no significant interaction among genotype, subunit and age ($F = 2.26$, d.f. = 2,22, $p = 0.0951$), indicating that similar changes in subunit expression occurred at each age (fig. 2). The significant interaction term was due to a statistically significant decrease in the mean percentage of neurons expressing the NR2A subunit at each age ($t = 4.16$, d.f. = 24, $p = 0.0004$). There was a trend for the decrease to be larger in the two older groups. There were no significant differences in the mean percent of neurons expressing NR1 or NR2B subunits.

Relation of Expression of NR2A to Different Subpopulations of Striatal Neurons
Since there was no significant interaction among genotype, subunit and age, neurons were pooled across age groups to determine whether the cells that lacked NR2A belonged to either subpopulation of SP- or ENK-expressing cells. The number of cells from each mouse was separated into subpopulations according to expression of SP and ENK and NR1, NR2A and NR2B subunits. There were no statistically significant differences in the proportion of cells expressing SP and NR1, NR2A or NR2B in WT mice (fig. 3). However, in R6/2 mice there was a statistically significant decrease in the proportions of cells expressing SP and NR2A compared to proportions expressing SP and NR1 or NR2B ($p < 0.001$ for the proportions expression SP/NR1 vs. SP/NR2A; $p < 0.001$ for the
proportions expressing SP/NR2B vs. SP/NR2A; ANOVA followed by Bonferroni t tests). Similarly, while there were no statistically significant differences in the proportion of cells expressing ENK and NR1, NR2A or NR2B in WT mice (fig. 3), in R6/2 mice there was a statistically significant decrease in the proportions of cells expressing ENK and NR2A compared to proportions expressing ENK and NR1 or NR2B (p < 0.001 for the proportions expressing ENK/NR1 vs. ENK/NR2A; p < 0.001 for the proportions expressing ENK/NR2B vs. ENK/NR2A; ANOVA followed by Bonferroni t tests). Furthermore, the ANOVA followed by the Bonferroni t tests indicated a statistically significant decrease between the proportions of cells from WT and R6/2 mice expressing ENK/NR2A (p < 0.001) but not between the proportion of SP-expressing cells in WT and R6/2 mice. Taken together these data indicate that NR2A expression is reduced in neurons that contain both SP and ENK in the R6/2 mice. The reduction is greater in cells that express ENK when compared only to the WTs.

**Semi-Quantitative RT-PCR**

Semi-quantitative RT-PCR reactions were performed in triplicate to determine changes in expression of NMDAR subunits. For this experiment total RNA was extracted from the whole striatum. Figure 4a shows sample bands from parallel PCR reactions comparing the expression of NR1, NR2A and NR2B and the housekeeping gene, β-actin in tissue from a WT and a transgenic mouse.
in the oldest age group. Figure 4b shows the ratio of gene expression in WT and R6/2 for the NMDARs with β-actin. Figure 4 corroborates the single-cell RT-PCR results, showing that there is a significant decrease in the levels of expression of NR2A in R6/2 compared to WT mice (p < 0.001 for the difference in ratios between WT and R6/2 for NR2A; ANOVA followed by Bonferroni t test). In addition, figure 4 also demonstrates that there is a statistically significant decrease in the ratio of NR2B in the R6/2 compared to WT mice (p = 0.006 for the difference in ratios between WT and R6/2 for NR2B; ANOVA followed by Bonferroni t test).

**Discussion**

The results of this study show, using both single-cell and semi-quantitative RT-PCR, that there are changes in the subunit composition of NMDARs in R6/2 transgenic mice. Furthermore, these changes are present by about 3 weeks of age. We observed a significant decrease in the percentage of medium-sized neurons expressing NR2A and this difference increased slightly with age. Semi-quantitative studies of striatal tissue mRNA confirmed the significant decrease in NR2A in the older age group and indicated in addition a significant decrease in NR2B. Single-cell studies showed that the decrease in NR2A occurred in cells that express SP or ENK and that the decrease was greater in the ENK-expressing cells when WT and R6/2 mice were compared. Others have reported that neurons expressing ENK are affected earlier than those expressing SP in the R6/2 [Menalled et al., 2000].

Although we did not detect significant changes in the proportion of cells expressing NR2B in single-cell RT-PCR experiments, there was a decrease in NR2B expression in the semi-quantitative experiment in the oldest group. These findings are not contradictory since the single-cell and tissue studies detect two different parameters of expression. The single-cell experiments show only the presence or absence of expression and not whether there is more or less of it present in the tissue. Thus it is quite conceivable that all or most cells express NR2B, but express less of it than in tissue from WT mice.

These results are consistent with previous findings from our laboratory showing a change in the postsynaptic NMDAR in R6/2 mice [Cepeda et al., 2001a]. Electrophysiological findings have shown an increased sensitivity of subpopulations of medium-sized neurons to activation of NMDARs in R6/2 mice [Levine et al., 1999; Cepeda et al., 2001a; Starling et al., 2005]. These cells also tend to have depolarized resting membrane potentials, which would facilitate NMDAR activation [Cepeda et al., 2001a; Klapstein et al., 2001]. Studies of protein expression using NR2A/NR2B antibodies also corroborate a decrease in NR2A and NR2B protein in the striatum of R6/2 mice [Cepeda et al., 2001a]. However, studies of the mRNA and protein levels of NR1 in HD have not always been in agreement. In the present study we report no significant changes in the expression of NR1 mRNA in the dorsolateral striatum. In contrast, we have previously reported that there is an increase in NR1 protein in R6/2 striatum compared to WT using immunohistochemical methods [Cepeda et al., 2001a]. It is possible that mRNA and protein expression do not always change in the same
direction. Other studies have reported no differences in the mRNA or protein levels of NR1 in the striatum of R6/2 mice [Luthi-Carter et al., 2003] or in YAC 46 and YAC 72 transgenic models of HD [Li et al., 2003]. A recent study indicated significant changes in NMDAR subunit RNA in the hippocampus of the R6/2 transgenic with minimal changes in the striatum, although NR2A was reduced at one age [Luthi-Carter et al., 2003]. It remains to be determined why many of the outcomes of these studies differ. Methodological differences may have contributed as other studies tended to examine binding, or used tissue homogenates. A potential problem in the present study that applies to single-cell RT-PCR studies is that the selection of healthy living striatal neurons may have biased against the collection of cells that were possibly already affected and did not survive the dissociation process. However, since changes also were observed in younger neurons, the potential for examining injured cells would be less likely in those groups. In conclusion, when taken together the outcomes of these many studies indicate that NMDAR changes are complex and we are just beginning to understand some of them and their associated mechanisms.

Studies on Mg\(^{2+}\) sensitivity in dissociated neurons support the hypothesis of postsynaptic changes in NMDARs. Recordings in acutely dissociated neurons demonstrate a decreased sensitivity to Mg\(^{2+}\) blockade of NMDA-activated currents in a subpopulation of R6/2 compared to WT medium-sized neurons [Starling et al., 2005]. This reduction in Mg\(^{2+}\) sensitivity was observed as early as 15 days of age, before any behavioral or biochemical changes could be seen in the mouse model. Similarly, the present study showed decreased expression of NR2A mRNA in the youngest group, which was slightly older than 15 days (19–25 days in the present experiments). A structural alteration in the NMDAR due to changes in subunit composition could affect the binding of Mg\(^{2+}\) to the channel [Kumar and Huguenard, 2003; Qian et al., 2005]. Furthermore, the decrease in blockade of NMDA channels by Mg\(^{2+}\) would result in an increased sensitivity of the channel to glutamate.

It has been suggested that presynaptic mechanisms such as altered release of glutamate from cortical afferents contribute to the dysfunction and the excitotoxicity-related death of medium-sized spiny neurons. In keeping with this hypothesis, there is a downregulation of transcription for genes for group II metabotropic glutamate receptors (mGluR2 and mGluR3) in the striatum and to a greater extent in the cortex of the R6/2 model [Cha et al., 1999]. Since mGluR2 is involved in the release of glutamate from corticostriatal terminals, its downregulation may lead to aberrant glutamate release. Moreover, we have reported transient increases in the frequency of large amplitude spontaneous excitatory postsynaptic currents in R6/2 mice at an age when overt behavioral symptoms begin, which would be in agreement with down-regulation of mGluRs 2 and 3 [Cepeda et al., 2003].

The presence of protein aggregates and nuclear inclusions in HD have several deleterious effects. Protein aggregates can bind to synaptic vesicles and inhibit the uptake of glutamate in vitro both in R6/2 mice and cell lines [Li et al., 2000]. The nuclear inclusions formed by the mutant HD protein have been shown to dysregulate gene transcription. Recent findings reveal that the mutant protein directly interferes with the binding of Sp1, a constitutively active regulatory protein that binds to GC boxes [Dunah et al., 2002]. Interestingly, studies also report the importance of Sp1 in transcriptional regulation of the NR2A subunit, which is decreased in the R6/2 model.

In addition, recent studies on excitotoxicity in HD suggest selectivity for NMDAR subunits. NMDAR-mediated increases in current amplitude and apoptosis have been reported in cell lines expressing full-length mutant huntingtin in combination with NR1/NR2B but not NR1/NR2A. In YAC transgenic mouse models expressing full-length mutant huntingtin, the excitotoxic death of neurons after intrastriatal injection of quinolinic acid, a relatively selective NMDA agonist, was abolished by an NR2B subtype-specific antagonist [Zeron et al., 2002]. Together, these findings support a role for NR2B subtype NMDAR activation as a potential trigger for selective neuronal degeneration in HD. As previously indicated, NMDARs containing NR2A are located mostly in the synapse whereas receptors containing NR2B are present at the synapse and extrasynaptically [Zeron et al., 2002]. A decrease in the NR2A/NR2B ratio may result in cells that are more vulnerable to excitotoxic death. The present findings do not necessarily contradict published reports of increased responsiveness to an NR2B antagonist in striatal neurons in YAC72 mice [Zeron et al., 2002; Li et al., 2003]. For example, if more cells make NMDA receptors with NR1 and NR2B subunits (since the proportion of cells containing NR2A subunits is decreased), one would expect NMDA currents to be more affected by an NR2B antagonist. In our single-cell and semi-quantitative RT-PCR experiments, we demonstrated a significant decrease in the expression of NR2A. A decrease in NMDARs at the synapse or an altered composition of the...
NMDARs could act synergistically with presynaptic changes to result in neuronal dysfunction.

In conclusion, the present results provide evidence to support the hypothesis of altered NMDAR subunit composition in striatal medium-sized neurons in R6/2 transgenic mice. Furthermore, these alterations may occur early in the development of the phenotype, even before overt behavioral effects are apparent. Changes in subunit composition of NMDAR postsynaptically may result in an enhanced responsiveness to glutamate, increasing vulnerability to excitotoxicity.

Acknowledgments

This work was supported by grants and contracts from the Heeditary Disease Foundation, the CURE Initiative, the USPHS NS 41574. We want to thank Donna Crandall and Carol Gray for the preparation of the illustrations.


