Altered Cortical Glutamate Receptor Function in the R6/2 Model of Huntington’s Disease

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INTRODUCTION

Huntington’s disease (HD) is a neurodegenerative disorder that includes motor and cognitive deficits (Jossiasssen et al. 1983; Thompson et al. 1988). An abnormal expansion of CAG repeats on the huntingtin (htt) gene is responsible for the disease (Nasir et al. 1996; Snell et al. 1993). In humans, mutated htt induces a preferential loss of medium-sized spiny neurons (MSSNs) of the striatum and, to a lesser extent, a loss of cortical neurons as the disease progresses (Albin et al. 1992; DiFiglia 1990; Graveland et al. 1993). In contrast to the human, most genetic mouse models of HD show behavioral and electrophysiological alterations in the absence of cell loss, suggesting that neuronal dysfunction is sufficient to produce HD symptoms (Levine et al. 2004). R6/2 mice are transgenic for exon 1 of the HD gene and exhibit a progressive neurological phenotype characteristic of juvenile HD (Davies et al. 1997; Mangiarini et al. 1996). In this model, cortical pyramidal neurons develop a late-onset degeneration and display morphological alterations such as decreases in somatic size and number of spines, suggesting that inputs to the cortex are altered (Klapstein et al. 2001; Laforet et al. 2001; Turmaine et al. 2000). In addition, striatal MSSNs in R6/2 mice display transient and progressive alterations in spontaneous excitatory synaptic activity that are likely to originate from changes in the corticostriatal pathway (Cepeda et al. 2003).

In the present study, we examined glutamate (AMPA and NMDA) receptor function and voltage-gated barium currents in pyramidal neurons of the sensorimotor cortex in the R6/2 model of HD at different time points of the disease. We found that glutamate receptor function in cortical pyramidal neurons was altered early in R6/2 mice and that changes in the cortex contrast from those occurring in striatal cells in R6/2 mice of the same age.

METHODS

Groups and cells

Experiments were performed on R6/2 transgenic mice and their wild-type (WT) littermate controls. Mice were obtained from our colony at UCLA. All experimental procedures were performed in accordance with the United States Public Health Service Guide for Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at UCLA. Three age groups of R6/2 mice were studied: 21 days (before overt behavioral symptoms), 40 days (when the behavioral phenotype begins), and 80 days (fully symptomatic). Forty-six mice in the three age groups were used (Table 1). In the 21-day group, NMDA and AMPA currents were recorded from the same cells to reduce the number of animals and provides the major glutamatergic input to other cortical areas and to subcortical areas such as the dorsolateral striatum (Cospito and Kultas-Illinsky 1981; Gubellini et al. 2004; McGeer et al. 1977; Reubi and Cuenod 1979). Alterations in glutamatergic cortical cell function induce corticocortical and corticostratial dysfunction, defective gating to the cortex and striatum, and could contribute to HD symptoms such as motor impairment and learning deficits (Abbruzzese and Berardelli 2003; Dunnett et al. 2005; Sapp et al. 1999).

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because we found in preliminary tests that exposure to either AMPA or NMDA did not alter average current amplitudes induced by the other agonist (Table 1). In the 40- and 80-day groups, we used different concentrations of agonists to determine the most effective concentration. Because our ejection system did not have enough capacity to perform dose–response curves for two agonists on the same neurons, cells were tested separately for AMPA and NMDA in these groups. Only data from cells for which access resistance values were <20 MΩ were included.

Dissociated cell preparation

Mice were anesthetized with halothane, transcardially perfused with 10 ml cold sucrose solution containing (in mM): 250 sucrose, 11 glucose, 15 HEPES, 1 NaHPO₄, 4 MgSO₄, and 2.5 KCl (pH 7.4, 300–310 mOsm), and decapitated. Brains were dissected and sliced in external recording solution contained Ba₂⁺, glycine, and 0.0003 tetrodotoxin (TTX, pH 7.4, 300–310 mOsm). The 0.1 CaCl₂, 23 glucose, and 15 HEPES (pH 7.4, 300–310 mOsm) and Louis, MO) bubbled with 95% O₂-5% CO₂ and supplemented with (in mM) 1 pyruvic acid, 0.005 glutathione, 0.1 N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid] (HEPES) 0.1 mM, and 1 kynurenatic acid until dissociation. After a 1-h incubation, somatosensory and motor areas of the cortex were dissected, placed in an oxygenated cell-stir chamber (Wheaton, Millville, NJ), and enzymatically treated for 25–30 min with papain (0.625 mg/ml, Calbiochem) at 35°C in a HEPES-buffered Hank’s balanced salt solution (HBSS, pH 7.4, 300–310 mOsm; Sigma). After enzymatic digestion, the tissue was rinsed with a low Ca²⁺ HEPES-buffered Na-isethionate solution containing (in mM): 140 Na⁺ isethionate, 2 KCl, 2 MgCl₂, 0.1 CaCl₂, 23 glucose, and 15 HEPES (pH 7.4, 300–310 mOsm) and mechanically dissociated with a graded series of fire-polished Pasteur pipettes. The cell suspension was plated into a Petri dish containing a HEPES-buffered salt solution consisting of (in mM): 140 Na⁺ Cl₂, 45 HEPES, 2 KCl, 2 MgCl₂, and 1 CaCl₂ (pH 7.4, 300–310 mOsm).

Whole cell voltage-clamp recordings

The internal pipette solution contained (in mM): 175 N-methyl-D-glucamine (NMDG), 40 HEPES, 2 MgCl₂, 10 ethylene glycol-bis (β-aminomethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), 12 phosphocreatine, 2 Na₃ATP, 0.2 Na₂GTP, and 0.1 leupeptin (pH 7.2–7.3, 265–270 mOsm). The Mg²⁺-free external solution consisted of (in mM): 135 NaCl, 20 CsCl, 5 BaCl₂, 10 glucose, 10 HEPES, 0.02 glycine, and 0.003 tetrodotoxin (TTX, pH 7.4, 300–310 mOsm). The external recording solution contained Ba²⁺, instead of Ca²⁺, to minimize the influence of Ca²⁺-mediated transduction systems. AMPA and NMDA receptors are permeable to Ca²⁺ and Ba²⁺ (Mayer and Westbrook 1987). Thus a portion of AMPA- and NMDA-mediated currents in these experiments is carried by Ba²⁺. The presence of Ca²⁺ and TTX in the external solution blocked some voltage-gated K⁺ and all Na⁺ channels, respectively.

Electrode resistance was typically 4–5 MΩ in the bath. After seal rupture, series resistance was compensated (70–90%) and periodically monitored. Signals were detected with an Axopatch 200A amplifier (Axon Instruments, Foster City, CA). Membrane capacitances and input resistances were measured by applying a 10-mV depolarizing step voltage command and using the membrane test function integrated in the pClamp8 software (Axon Instruments). Capacitance and input resistance were measured to compare membrane properties between neurons from WTs and R6/2s.

Drug application

Drugs were applied through an array of application capillaries positioned 500–600 μm from the cell using a pressure-driven fast perfusion system. Solution changes were performed by changing the position of the array with a DC drive system controlled by a SF-77B perfusion system (Warner Instruments, Hamden, CT) synchronized by pClamp. Solution changes were complete within <100 ms. Agonists, antagonists, and modulators were applied for 3 s every 10 s. Voltage-gated Ba²⁺ currents were evaluated by measuring the Ba²⁺ peak current evoked by step voltage commands (−80 to −10 mV, 1 s). Responsiveness of cells to 100 μM AMPA in the absence or presence of 10 μM cyclothiazide (CTZ, inhibitor of AMPA receptor desensitization) were examined at a holding potential of −80 mV to increase the driving force. The non-NMDA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM) was applied to confirm the specificity of AMPA-induced currents. Responsiveness of cells to 100 μM NMDA and their sensitivity to 50 μM Mg²⁺ were examined at four different holding potentials: −70, −40, −20, and 0 mV. The NMDA-receptor–specific antagonist 2-amino-5-phosphononovalerate (APV, 50 μM) was applied to confirm the specificity of NMDA-induced currents. We paid special attention to AMPA application to avoid the variability introduced by a relatively slow application system to measure a fast peak. Before recording the traces, we positioned the application capillaries at an optimal distance to induce the maximal peak current and we applied four times more pressure than that for NMDA currents.

Data analysis

In the text, values are presented as means ± SE. Data analyses were performed with Origin (Microcal Software, Northampton, MA) and pClamp software. Electrophysiological membrane properties and AMPA and NMDA current characteristics were compared using a two-way ANOVA followed by Bonferroni t-test using SigmaStat software (SPSS, Chicago, IL). We used two-way repeated-measures ANOVAs followed by Bonferroni t-tests to compare NMDA current characteristics among different holding voltages and AMPA current characteristics before and after CTZ application in WT and R6/2 cells. To measure the desensitization time constants, AMPA current decay rates were fit using a single exponential. We chose to fit between the peak and at 300 ms after the peak because at 300 ms AMPA currents reached a steady state. The Simple PCI image analysis system software (Compix, Cranberry Township, PA) was used to measure cell somatic area to compare cell sizes statistically among groups using a two-way ANOVA followed by Bonferroni t-test. Differences were considered statistically significant when P < 0.05.

RESULTS

Membrane properties, cell morphology, and Ba²⁺ currents

Only data from pyramidal-shaped cells with clear apical and basilar dendrites were included in the present study (Figs. 1A

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TABLE 1. Number of WT and R6/2 animals in each age group (mean ± SE) and number of recorded cells for AMPA and NMDA currents

<table>
<thead>
<tr>
<th></th>
<th>Mean Age, days</th>
<th>Cells</th>
<th>Mean Age, days</th>
<th>Cells AMPA/NMDA</th>
<th>Mean Age, days</th>
<th>Cells AMPA/NMDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>21 Days</td>
<td>WT 22.8 ± 0.7 n = 5</td>
<td>29</td>
<td>R6/2 22.2 ± 0.6 n = 5</td>
<td>22</td>
<td>28/19</td>
<td>78.1 ± 1.5 n = 8</td>
</tr>
<tr>
<td></td>
<td>40 Days</td>
<td></td>
<td>40.4 ± 1.2 n = 10</td>
<td>32/16</td>
<td>77.3 ± 1.6 n = 10</td>
<td>27/23</td>
</tr>
</tbody>
</table>

J Neurophysiol • VOL 95 • APRIL 2006 • www.jn.org
and 5A). Most pyramidal neurons had capacitances ($C_m$) between 10 and 20 pF. Occasionally, larger cells, presumably from layer V, were found and had $C_m$ values $>25$ pF. Only data from cells for which $C_m$ values were $<25$ pF were included to omit obvious large cells. In the 40-day group, $C_m$ was significantly smaller in the R6/2 transgenic cells, suggesting a smaller size for pyramidal neurons from R6/2 animals, which was verified by somatic area measures (Table 2). There was no significant change in $C_m$ in the 21- and 80-day R6/2 cells. Input resistance ($R_m$) was significantly higher in R6/2 cells in the 40-day group, whereas there was no significant change in the 21- and 80-day groups. Somatic areas were similar in R6/2 and WT cells at 21, 40, and 80 days. In the 21- and 40-day groups, there were no statistically significant differences in Ba$_{2+}$ currents and current densities between WT and R6/2. In contrast, in the 80-day group, Ba$_{2+}$ currents and current densities were significantly larger in R6/2 cells (Table 2).

### Table 2. Membrane properties and morphological characteristics of WT and R6/2 cortical pyramidal neurons at 21, 40, and 80 days of age

<table>
<thead>
<tr>
<th></th>
<th>Capacitance, pF</th>
<th>Input Resistance, GΩ</th>
<th>Somatic Area, µm$^2$</th>
<th>Ba$_{2+}$ Current Density, pA/pF</th>
</tr>
</thead>
<tbody>
<tr>
<td>21 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>19.7 ± 0.9</td>
<td>1.3 ± 0.2</td>
<td>135 ± 8</td>
<td>18.7 ± 2.5</td>
</tr>
<tr>
<td>R6/2</td>
<td>17.9 ± 0.9</td>
<td>1.7 ± 0.2</td>
<td>118 ± 7</td>
<td>20.9 ± 3.8</td>
</tr>
<tr>
<td>40 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>16.2 ± 0.6</td>
<td>1.1 ± 0.1</td>
<td>142 ± 6</td>
<td>16.9 ± 3.2</td>
</tr>
<tr>
<td>R6/2</td>
<td>13.4 ± 0.7*</td>
<td>1.6 ± 0.1*</td>
<td>136 ± 6</td>
<td>19.8 ± 2.2</td>
</tr>
<tr>
<td>80 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>16.3 ± 0.6</td>
<td>1.1 ± 0.1</td>
<td>130 ± 6</td>
<td>15.3 ± 1.9</td>
</tr>
<tr>
<td>R6/2</td>
<td>15.6 ± 0.6</td>
<td>1.2 ± 0.1</td>
<td>127 ± 5</td>
<td>25.8 ± 3.0*</td>
</tr>
</tbody>
</table>

*Statistical significance using a two-way ANOVA followed by Bonferroni t-test, $P < 0.05$. 

**AMPA currents**

Different concentrations of AMPA (10, 100, and 1,000 µM) were applied in 40-day WT ($n = 12$) and R6/2 ($n = 10$) cells to determine the concentration–response function. Application of 10 µM AMPA induced a nondesensitizing current, whereas 1,000 µM AMPA induced a fast desensitizing current that decreased to a steady-state plateau in WT and R6/2 cells. The response to AMPA was smaller in the R6/2 transgenic cell. The bar graph shows the mean peak current densities induced by application of different concentrations of AMPA. At all concentrations, AMPA current densities were smaller in R6/2 cells. Statistical significance using a 2-way ANOVA followed by Bonferroni t-test, $P < 0.05$. 

![FIG. 1. Representative cortical pyramidal neurons from WT and R6/2 transgenic mice at 40 days and their α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)–induced currents. A: cortical pyramidal neurons were recognized by their pyramidal shape and the presence of an apical dendrite. B: cortical pyramidal neurons were recognized by their pyramidal shape and the presence of an apical dendrite. C: in this example, in both genotypes, 10 and 100 µM AMPA induced a current showing a small peak or no peak followed by a steady-state, whereas 1,000 µM AMPA currents showed a fast desensitizing peak followed by a steady state. Traces show that AMPA currents were smaller in the R6/2 transgenic cell. C: bar graph shows the mean peak current densities induced by application of different concentrations of AMPA. At all concentrations, AMPA current densities were smaller in R6/2 cells. *Statistical significance using a 2-way ANOVA followed by Bonferroni t-test, $P < 0.05$.](http://jn.physiology.org)
We measured the peak and the steady-state amplitudes of AMPA currents at 100 μM concentration in each age group (Fig. 2). R6/2 neurons displayed significantly smaller AMPA (peak and steady-state) current amplitudes and densities compared with WT cells at 40 days. At 21 days, AMPA (peak and steady-state) amplitudes and densities were not statistically different, although they tended to be smaller in R6/2 cells. At 80 days, AMPA peak current amplitudes and densities, and steady-state current amplitudes and densities, were similar between WT and R6/2 cells (Fig. 2). Desensitization rates (measured 300 ms after the peak) were significantly shorter in R6/2 compared with WT cells at 21 days \([48.7 \pm 3.5 \text{ ms (WT)} \text{ vs. } 34.4 \pm 4.3 \text{ (R6/2)}]\) and at 40 days \([40.5 \pm 3.0 \text{ ms (WT)} \text{ vs. } 29.4 \pm 2.9 \text{ (R6/2)}]\); two-way ANOVA followed by Bonferroni \(t\)-test, \(P < 0.05\). However, there were no statistically significant differences between WT and R6/2 cells at 80 days \([47.7 \pm 4.0 \text{ ms (WT)} \text{ vs. } 40.7 \pm 2.8 \text{ (R6/2)}]\).

To determine whether the differences in AMPA currents in WT and R6/2 cells could result from differential splicing of the subunits into flip and flop forms, we measured the mean steady-state to peak ratio for AMPA currents. The ratio was significantly smaller in R6/2 compared with WT cells at 21 days \([0.62 \pm 0.03 \text{ (WT)} \text{ vs. } 0.50 \pm 0.03 \text{ (R6/2)}]\) and 40 days \([0.50 \pm 0.03 \text{ (WT)} \text{ vs. } 0.39 \pm 0.03 \text{ (R6/2)}]\); two-way ANOVA followed by Bonferroni \(t\)-test, \(P < 0.05\) but not at 80 days \([0.54 \pm 0.03 \text{ (WT)} \text{ vs. } 0.51 \pm 0.03 \text{ (R6/2)}]\).

Coapplication of 10 μM CTZ reduced the desensitization and potentiated AMPA-induced currents in all WT and R6/2 cells at each age. AMPA peak currents were 6–8 times larger when CTZ and AMPA were coapplied. Furthermore, currents did not exhibit the fast desensitizing peak component observed with AMPA alone (Fig. 3A). At 21 and 40 days, the AMPA peak current amplitudes and densities induced in the presence of CTZ were significantly smaller in R6/2 compared with WT cells (Fig. 3B). Furthermore, two types of responses to CTZ were observed in WT and R6/2 cells. Although CTZ reduced the fast desensitization of the AMPA current, there was still a slow desensitization (Fig. 3A, right trace) in about 50% of the WT cells (43% at 21 days, 44% at 40 days, and 50% at 80 days). In the R6/2 group we also found a slow desensitization of AMPA currents in the presence of CTZ at all ages (89% at 21 days, 87% at 40 days, and 50% at 80 days) (Fig. 3C). The differences in frequency between R6/2 and WT for the slow desensitizing component were statistically significant at 21 and 40 days \((\chi^2, P < 0.001 \text{ at both ages})\). At 80 days there was no
difference in the percentage of cells showing desensitizing currents in the presence of CTZ in WT and R6/2 cells.

We then classified WT and R6/2 cells in two groups, depending on the presence of the AMPA desensitizing component in the presence of CTZ (see Fig. 3A), and found differences in current characteristics. Currents induced by AMPA alone were significantly larger if the subsequent CTZ response showed no desensitization. This was reflected by smaller AMPA peak current amplitudes and densities for currents with the desensitizing component at all ages (Fig. 4A).

Similarly, in WT and R6/2 cells, in all age groups, AMPA currents modulated by CTZ were significantly larger when the currents did not desensitize (Fig. 4B).

**NMDA currents**

We initially tested increasing concentrations of NMDA on pyramidal neurons in the absence of Mg$^{2+}$ in cells from 40-day-old mice. Application of NMDA (1–1,000 μM, 3-s duration, $V_{\text{hold}} = -70$ mV) induced inward currents that increased in a concentration-dependent manner. At concentrations of ≥30 μM, the NMDA response consisted of a fast peak, followed by a slowly desensitizing component (Fig. 5B). EC$_{50}$ for WT cells at 40 days was 53.0 ± 4.6; (n = 11) and was not different from R6/2 cells (45.0 ± 3.8; n = 12). NMDA-induced peak current amplitudes and densities for each NMDA concentration were similar in WT and R6/2 cells (Fig. 5C).

Application of 50 μM of the specific NMDA antagonist APV blocked 85–90% of 100-μM NMDA currents in WT and R6/2 transgenic cells (data not shown). In subsequent experiments, we used a nonsaturating concentration (100 μM) of NMDA to test Mg$^{2+}$ sensitivity. We show data only for 70 and 40 mV holding potentials because differences between WT and R6/2 were significant at those two voltages.

In the absence of Mg$^{2+}$, NMDA current amplitudes and densities were dependent on voltage and decreased at −40 mV compared with −70 mV in WT and R6/2 cells at all ages (Fig. 6). At 21 days, 100 μM NMDA induced significantly smaller...
peak currents in R6/2 compared with WT cells at −70 and −40 mV holding potentials. Current densities were significantly smaller in R6/2 cells at −70 mV only (Fig. 6). At 40 days NMDA peak currents and densities were similar in WT and R6/2 cells, although peak currents tended to be smaller in R6/2 cells at −70 and −40 mV. There were no differences in NMDA currents and current densities between WT and R6/2 cells at 80 days.

Coapplication of 50 μM Mg²⁺ with 100 μM NMDA decreased currents in WT and R6/2 cells in all groups (Fig. 7). At 21 days, when Mg²⁺ was added, NMDA currents and current densities were smaller in R6/2 cells at −70 mV. At 40 days, currents and current densities in the presence of Mg²⁺ were significantly smaller in R6/2 compared with WT cells at both holding potentials. At 80 days, both NMDA currents and current densities were similar in WT and R6/2 cells at both holding potentials when Mg²⁺ was added. As expected, Mg²⁺ inhibition was voltage dependent and decreased with the less-negative holding potentials at all ages (Fig. 7C). At 21 days, the percentage Mg²⁺ block was similar in WT and R6/2 transgenic cells at −70 and −40 mV. However, at 40 days, Mg²⁺ block was significantly larger in R6/2 cells at both holding potentials. In the 80-day-old group, there was a significantly larger Mg²⁺ block at the −70 mV holding potential in R6/2 cells.

**Discussion**

The present results indicate that cortical pyramidal neurons from the R6/2 transgenic model of HD have decreased glutamate receptor-mediated currents as early as 21 days of age. These changes include smaller AMPA and NMDA currents, larger Mg²⁺ block indicating smaller NMDA currents in the presence of Mg²⁺, and more cells displaying desensitizing AMPA/CTZ currents. Changes were biphasic and seemed to be regulated by age because most of the early changes were not present at 80 days. Interestingly, Ba²⁺ peak currents were larger in R6/2 cells from 80-day-old mice only. This suggests that a complex interplay of ligand- and voltage-gated channel alterations occurs throughout the evolution of the disease, leading to different symptoms. These changes are region and time specific because they are different in the cerebral cortex and the striatum.

**Decreased glutamate currents**

Application of glutamate receptor agonists onto dissociated cells in the whole cell patch-clamp configuration allows the study of postsynaptic receptor function in isolation. Thus the decreases in AMPA and NMDA currents we observed are most likely attributable to altered receptor properties. However, it
may be argued that removal of most of the dendrites could affect the experimental outcome. This is unlikely because the cells we selected had similar dendritic fields. A limitation of our application system is the reduced speed that could introduce variability depending on the perfusion rate. Despite the fact that we maximized AMPA application (see METHODS), it is possible that we missed some of the fast AMPA peak, which could explain the relatively high variability in AMPA peak current amplitudes compared with NMDA. Nevertheless, we found significant differences in peak current, desensitization, and steady-state to peak current ratios consistent with a different subunit composition. Moreover, we confirmed smaller AMPA currents using CTZ, a compound that blocks AMPA peak desensitization.

The subunit composition of NMDA and AMPA receptors determines many of the properties of the currents. AMPA receptors are composed of four subunits (GluR1–4). Each subunit exists in the form of two splice variants, flip and flop, affecting the kinetics of the currents. Activation of AMPA receptors containing flip subunits induces currents with slower kinetics compared with receptors containing flop subunits (Partin et al. 1995; Vorobjev et al. 2000). In addition, CTZ reduces AMPA receptor desensitization differently, depending on the presence of flip and flop variants. In recombinant systems expressing flip subunits AMPA + CTZ induces a nondesensitizing current, whereas in cells expressing flop subunits AMPA + CTZ currents have a desensitizing component (Partin et al. 1994, 1995). In the present study, we found that...
more pyramidal neurons in the WT groups displayed non-desensitizing currents, consistent with previous studies describing the preferential expression of the flip form in cortical pyramidal neurons (Lambolez et al. 1996). The differences observed in R6/2 transgenic cells seem to be the result of a change in AMPA receptor flip/flop subunit composition because more cells from the R6/2 transgenic group displayed: 1) desensitizing AMPA/CTZ currents, 2) smaller AMPA/CTZ currents, 3) smaller AMPA currents with faster desensitization time constants, and 4) smaller steady-state to peak current ratio. We also found that the amplitude of desensitizing AMPA/CTZ currents was smaller than the amplitude of non-desensitizing currents, which explains the overall decreased amplitude in AMPA currents observed in R6/2 cells.

Likewise, NMDA receptors are composed of several subunits: two NR1 along with two or three NR2 subunits that confer Mg$^{2+}$ sensitivity to the receptor (Behe et al. 1995; Hollmann and Heinemann 1994; Ishii et al. 1993; Monyer et al. 1994). A change in the subunit composition could alter NMDA currents and Mg$^{2+}$ sensitivity in the R6/2 model. Previous studies examining NMDA currents in striatal MSSNs in this model found larger currents that could arise from a change in expression of NR2 subunits (Cepeda et al. 2001; Starling et al. 2005). It is still unclear how the subunit composition can alter NMDA receptor currents and Mg$^{2+}$ sensitivity because some studies reported a decrease of NR2A/B subunits, whereas others demonstrated increased responses to NR2B antagonists (Ali and Levine 2006; Cepeda et al. 2001; Li et al. 2003; Zeron et al. 2002).

Smaller AMPA and NMDA currents could also be the consequence of fewer receptors present at the cellular surface. It has been demonstrated that cortical pyramidal neurons from R6/2 transgenic mice show decreases in somatic size, the extent of the dendritic field, and the number of spines (Klapstein et al. 2001). We observed that the capacitance was smaller in the transgenic neurons at 21 and 40 days, which could partially account for smaller glutamate currents. However, the differences in NMDA and AMPA currents remained when we computed current density, indicating that alterations did not arise solely from differences in cell size. Instead, a decrease in the number of AMPA and NMDA receptors in R6/2 cells could explain smaller currents. In fact, in R6/2 mice and HD in general, polyglutamine expansion impairs the ability of htt to bind to the postsynaptic density 95 protein and other plasma membrane–associated proteins that help anchor NMDA and AMPA receptors to the membrane and more.

**FIG. 6.** Graphs showing mean peak currents (±SE) and mean peak current densities (±SE) evoked by 100 μM NMDA in the absence of Mg$^{2+}$ in 21-, 40-, and 80-day-old cortical pyramidal neurons. A: at 21 days mean NMDA peak currents were significantly smaller in R6/2 cells at −70 and −40 mV holding potentials. There were no significant differences at 40 and 80 days between WT and R6/2 mean peak currents. B: at 21 days the mean peak current density at −70 mV was significantly smaller in R6/2 vs. WT cells. At 40 and 80 days there were no significant differences in mean peak current densities at any holding potential. *Statistical significance using a 2-way ANOVA followed by Bonferroni t-test, P < 0.05.
specifically to the synapse (Jarabek et al. 2004; Luthi-Carter et al. 2003; Sun et al. 2001).

At a more integrative level, the cerebral cortex is interconnected with basal ganglia structures, and a function of these structures is to modulate sensory cortical input (Schwarz et al. 1992). A decrease in glutamate receptor function in the cortex could therefore result from early alterations in the striatum. Because cortex and striatum are so closely interconnected, alterations in the cortex could also have an impact on the striatum. To determine which structures htt alters first, it is necessary to study the disease at very early time points. New lines of conditional mice expressing the mutated htt in certain cell types will certainly help to clarify the sequence of events leading to pathology (Gu et al. 2005).

**FIG. 7.** Bar graphs showing mean peak currents (±SE), current densities (±SE) evoked by 100 µM NMDA in the presence of 50 µM Mg²⁺, and mean percentage block by 50 µM Mg²⁺ (±SE) in cortical pyramidal neurons from each age group. **A,** at 21 days mean NMDA peak currents were significantly smaller in R6/2 transgenic cells at the −70 mV holding potential. At 40 days they were smaller in R6/2 transgenic cells at −70 and −40 mV holding potentials. There were no significant differences at 80 days between WT and R6/2 mean peak currents. **B,** R6/2 mean peak current densities were significantly smaller than WT values at −70 mV in the 21- and 40-day-old groups. At 40 days, the peak current densities in R6/2 cells were also significantly smaller than WT at −40 mV. At 80 days, peak current density values were similar in WT and R6/2 cells. **C,** at 21 days, the percentage block was similar in WT and R6/2 cells. At 40 days, it was significantly larger in R6/2 compared with WT cells at −70 and −40 mV. At 80 days, the increase was significant only at −70 mV. *Statistical significance using a 2-way ANOVA followed by Bonferroni t-test, *P* < 0.05.
Consequences of smaller cortical NMDA and AMPA currents on projection structures

A decrease in cortical AMPA and NMDA receptor activation could have multiple consequences on cortical output structures. Although in the present study we do not know whether recorded cortical pyramidal neurons projected to the striatum, it is likely that at least a subset of cells were corticostriatal neurons. Decreased glutamate signaling in the cortex could therefore explain some of the changes seen in the striatum of R6/2 mice. More specifically, it is possible that a decrease in cortical AMPA receptor activation leads to morphological and functional striatal alterations. Activation of AMPA receptors upregulates expression of brain-derived neurotrophic factor (BDNF), which has a role in neuroprotection and synaptic plasticity on projection neurons (Lauterborn et al. 2000; Levine et al. 1998; Wu et al. 2004). When cortical BDNF is downregulated, it leads to striatal dendrite deficits, increased susceptibility of MSSNs to excitotoxic insults, and to HD pathlogy (Baquet et al. 2004; Canals et al. 2004). Decreased spontaneous excitatory postsynaptic currents (EPSCs) in striatal MSSNs occur in R6/2 mice (Cepeda et al. 2003). Alterations of spontaneous activity observed in the striatum had a presynaptic component because they remained after addition of TTX. A lack of BDNF could indirectly induce decreased activity along the corticostriatal pathway because BDNF regulates expression of AMPA receptors and increases miniature EPSCs (Bolton et al. 2000; Desai et al. 1999; Narisawa-Saito et al. 1999). Abnormal cortical function is likely to trigger a failure of the striatum to properly integrate inputs and could contribute to the phenotype in HD.

AMPA and NMDA receptor activation plays an important role in cortical long-term potentiation and depression (LTP and LTD) (Aroniadou and Teyler 1991; Johnston et al. 1992). LTD and LTD can be induced in sensorimotor cortex and are important for learning and memory processes, synaptic plasticity, and maturation of synapses on projection neurons (Bindman et al. 1988; Conrad et al. 1999). The decreases of glutamatergic responses we observed in the cortex might induce deficits in synaptic plasticity and memory in R6 transgenic mice (Cybulaska-Klosowicz et al. 2004; Murphy et al. 2000).

Comparison with previous studies

The fact that pyramidal neurons from R6/2 transgenic mice display smaller NMDA currents could explain why cortical neurons of R6/2 mice are not more vulnerable to kainate or NMDA exposure (Snider et al. 2003). Our finding that cortical pyramidal neurons from R6/2 transgenic mice display smaller AMPA currents correlates with previous clinical studies that found decreased AMPA binding in the frontal cortex of HD patients (Wagster et al. 1994). Functionally, a decrease in glutamatergic cortical function could help explain the decreased somatosensory-evoked potentials recorded in the cortex of HD patients and mice (Noth et al. 1984; Schwarz et al. 1992; Topper et al. 1993). If reduced cortical glutamatergic currents translate into less firing, our findings would correlate with the decrease in spontaneous EPSCs observed in MSSNs in R6/2 mice (Cepeda et al. 2003). Such alterations in the cortex and its projection structures could thus explain the surprising resistance of R6/1 and R6/2 mice to excitotoxic agents (Hansson et al. 1999; Morton and Leavens 2000). Hippocampal and striatal cell death induced by quinolinic or kainic acid injection requires an intact network (Koehler et al. 1978; McGeer et al. 1978). R6/2 mice develop less cell damage after kainate injection but also develop fewer seizures, reflecting decreased connectivity between hippocampus and cortex (Morton and Leavens 2000). In this case, the decreased connectivity of R6/2 hippocampal or striatal cells would protect them against repeated activation induced by excitotoxins in vivo.

An important point is that there is a regional specificity in NMDA receptor function in R6/2 mice. In contrast to cortical pyramidal cells, MSSNs from R6/2 mice display larger NMDA currents very early in development and at later stages (15, 21, and 40 days) and show more susceptibility to excitotoxicity (Cepeda et al. 2001; Laforet et al. 2001; Levine et al. 1999; Starling et al. 2005). Although synaptic activity is decreased, it is possible that extrasynaptic receptors are upregulated in the striatum because deafferentation of the striatum induces a long-term upregulation of glutamate receptors (Wullner et al. 1994). Bath application of NMDA activates synaptic and extrasynaptic receptors and could explain why striatal cells display larger NMDA currents. Although the changes in NMDA currents go in opposite directions in the cortex and the striatum, both structures are affected early in the R6/2 model.

The present study also found that changes in NMDA and AMPA currents were no longer present at 80 days. Sampling of the cells could explain this effect. In dissociated preparations, it is often more difficult to obtain healthy cells from R6/2 compared with WT mice, especially at 80 days when the phenotype is severe. If the HD gene affects neurons expressing certain types of receptors, it is possible that those neurons do not survive the dissociation procedure. However, we were able to find that Ba2+ currents were increased in 80-day R6/2 cells but unchanged at other ages, suggesting that cortical cells might be more excitable at that age. In support of this idea, sensitivity to kainate-induced seizures varies with age in R6/2 mice, with an increase in the degree of seizure severity at 60 days compared with 21 days (Morton and Leavens 2000). Seizures are also observed in patients with juvenile HD and at late stages of neurodegenerative disorders, presumably because the loss of cells or connections induces reorganization leading to abnormal networks (Landau and Cannard 2003; Seneca et al. 2004). These findings indicate that “biphasic” effects occur and could be either the result of compensatory mechanisms to a decreased excitatory function or the consequence of the progression of the disease.

In conclusion, we found evidence for early decreased glutamate receptor function in cortical pyramidal cells from R6/2 mice. Reduced or abnormal gating of cortical output structures might explain some features of the HD phenotype, especially cognitive deficits. Our findings suggest that cortical dysfunction could be considered an early symptom of HD and that treatments should target regions other than the striatum. For example, treatment of cognitive deficits could involve the use of AMPA modulators selectively increasing AMPA currents (O’Neill et al. 2004). The fact that cortical glutamatergic function is altered early, along with other studies showing early alterations in the striatum in the R6/2 model, suggests that treatment should begin well before overt symptoms appear.


