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NMDA Receptor Losses in Putamen from Patients with Huntington's Disease

ANNE B. YOUNG,* J. TIMOTHY GREENAMYRE, ZANE HOLLINGSWORTH, ROGER ALBIN, CONSTANCE D'AMATO, IRA SHOULSON, JOHN B. PENNEY

N-Methyl-D-aspartate (NMDA), phencyclidine (PCP), and quisqualate receptor binding were compared to benzodiazepine, γ-aminobutyric acid (GABA), and muscarinic cholinergic receptor binding in the putamen and cerebral cortex of individuals with Huntington's disease (HD). NMDA receptor binding was reduced by 93 percent in putamen from HD brains compared to binding in normal brains. Quisqualate and PCP receptor binding were reduced by 67 percent, and the binding to other receptors was reduced by 55 percent or less. Binding to these receptors in the cerebral cortex was unchanged in HD brains. The results support the hypothesis that NMDA receptor–mediated neurotoxicity plays a role in the pathophysiology of Huntington's disease.

HUNTINGTON'S DISEASE (HD) IS AN autosomal dominant inherited disorder characterized by progressive cognitive decline and involuntary movements starting in mid-life (*1*). Genetic linkage studies have localized the HD gene to the tip of chromosome 4, but the actual gene has not been isolated, and the HD biochemical defect is still unknown (*2*). Pathologically, HD is characterized by extensive neuronal loss in the caudate nucleus and putamen, and relative sparing of neurons in the rest of the brain (*3*). Striatal afferent pathways are largely spared. Among the neurochemical correlates of striatal neuron loss are decreases in striatal glutamate decarboxylase (GAD) activity, GABA, and substance P (*4*). Kainate, the glutamate agonist and neurotoxin, produces a loss of GABA and GAD when it is injected into rodent striata; afferent terminals and fibers of passage are spared. It was hypothesized that HD may be due to a genetic susceptibility to an exogenous or endogenous neurotoxin (*5*). The kainate model for HD became less attractive, however, when kainate was found to destroy all striatal neurons, whereas, in HD striatum, certain neurons were spared (*6*). Striatal injections of quinolinic acid, an endogenous neurotoxin, produced both the GABA losses and the selective sparing seen in HD (*7*). Although these findings have been challenged (*8*), studies in rodents and primates support the original observation (*9*). Quinolinic acid exerts its effects through a subtype of excitatory amino acid receptor, the *N*-methyl-D-aspartate (NMDA) receptor; NMDA receptor antagonists block quinolinate neurotoxicity (*9, 10*).

If NMDA receptors are involved in the pathogenesis of HD, cells with high densities of NMDA receptors should be preferentially lost in HD striatum, and NMDA receptor density should decline concommitantly. We examined this possibility by measuring NMDA and other receptors in cerebral cortex and putamen from HD brains.

Blocks of tissue were cut from frozen coronal sections through midputamen and globus pallidus of HD and control brains. The blocks included insular cortex and were coded and assayed by persons blind to the clinical diagnosis (*11*). In initial experiments, [^{3}H]glutamate binding to NMDA-sensitive and quisqualate-sensitive binding sites was measured. Sections from seven HD and six control brains (*11*) were washed with cold buffer for 30 min and then incubated in 20 n*M* [^{3}H]glutamate and 50 m*M* tris-HCl buffer, *p*H 7.2, containing 2.5 m*M* $CaCl_2$, with or without 100 μ*M* NMDA, 2.5 μ*M* quisqualate, 100 μ*M* NMDA plus 2.5 μ*M* quisqualate, or 1 m*M* glutamate as a blank (*12*).

[^{3}H]Flunitrazepam binding to benzodiazepine receptors, [^{3}H]muscimol binding to GABA receptors, and [^{3}H]quinuclidinyl benzilate binding to muscarinic cholinergic receptors were measured in the same group of brains (*11, 13*). All sections were placed in x-ray cassettes with appropriate standards, apposed to tritium-sensitive Ultrofilm ^{3}H (LKB), exposed for 1 to 6 weeks, and developed and analyzed by computer-assisted densitometry (*12, 13*). Five to eight readings in each area were averaged. Insular cortex and putamen were examined; caudate nucleus was too small to analyze reliably.

In control putamen, NMDA (100 μ*M*) competed for only 15% of total glutamate binding in tris-HCl–$CaCl_2$ buffer; quisqualate (2.5 μ*M*) competed for 35% (*14*). In cerebral cortex, 25% of total glutamate binding was NMDA sensitive and 50% was quisqualate sensitive. In HD putamen, both total glutamate and quisqualate-sensitive binding were reduced 67% in HD brains compared to control. The NMDA-sensitive binding was reduced 95% in HD compared to controls (Fig. 1). In this group of brains, there were significant but smaller decreases in muscarinic cholinergic (50%), $GABA_A$ (55%), and benzodiazepine receptors (55%) in HD putamen. The decreases in NMDA receptors were not statistically significant as compared to other receptors [$P < 0.07$, Kruskal-Wallis nonparametric one-way analysis of variance (ANOVA)]. Insular cortex receptor binding for all ligands was normal in HD brains.

Because only 15% of glutamate binding in tris-HCl–$CaCl_2$ buffer was sensitive to NMDA, we examined an additional five HD and six control brains (*11*) with the assay described above, as well as with two more specific assays for the NMDA receptor complex. In one assay for NMDA receptors,

A. B. Young, J. T. Greenamyre, Z. Hollingsworth, R. Albin, J. B. Penney, Department of Neurology, University of Michigan, Ann Arbor, MI 48109.
C. D'Amato, Department of Pathology, University of Michigan, Ann Arbor, MI 48109.
I. Shoulson, Department of Neurology, University of Rochester, Rochester, NY 14642.

*To whom correspondence should be addressed at Neuroscience Building, 1103 East Huron Street, Ann Arbor, MI 48104.

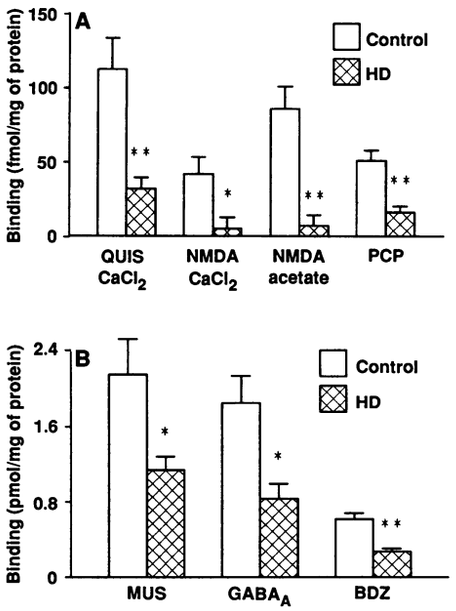

Fig. 1. Histograms of receptor binding in HD putamen. (**A**) Quisqualate-sensitive (QUIS $CaCl_2$) and NMDA-sensitive (NMDA $CaCl_2$) binding was measured with 20 n*M* [^{3}H]glutamate in 50 m*M* tris-HCl and 2.5 m*M* $CaCl_2$. Quisqualate-sensitive and NMDA-sensitive binding was determined by subtracting from total glutamate binding the amount displaced by 2.5 μ*M* quisqualate and 100 μ*M* NMDA, respectively (*12*). HD brains, $n = 12$; control brains, $n = 11$. NMDA receptors (NMDA acetate) were also measured in 20 n*M* [^{3}H]glutamate and 50 m*M* tris-acetate with 2.5 μ*M* quisqualate (*15*). Nonspecific binding was determined with 1 m*M* glutamate. HD brains, $n = 5$; control brains, $n = 6$. PCP receptors were measured with 40 n*M* [^{3}H]TCP in 50 m*M* tris-acetate buffer and 1 m*M* Mg acetate. Nonspecific binding was determined in 10 μ*M* TCP (*15*). HD brains, $n = 5$; control brains, $n = 6$. (**B**) Muscarinic cholinergic receptors (MUS) were measured with 1 n*M* [^{3}H]quinuclidinylbenzilate in 50 m*M* tris-HCl and nonspecific binding assessed with 1 μ*M* atropine. This assay measures the number of binding sites for the muscarinic antagonist. Benzodiazepine receptors (BDZ) were measured with various concentrations (2 to 150 n*M*) of [^{3}H]flunitrazepam in 50 m*M* tris-HCl; 5 μ*M* clonazepam was used as a blank, and saturation curves were carried out to measure the number of binding sites. $GABA_A$ receptors were measured with various concentrations (5 to 150 n*M*) of [^{3}H]muscimol in 50 m*M* tris-citrate; 100 μ*M* GABA was used as a blank, and saturation curves were analyzed for determination of the number of binding sites. The above methods have been described in (*13*). HD brains, $n = 7$; control brains, $n = 6$. Values in both (A) and (B) represent mean ± SEM. $*P < 0.04$, $**P < 0.004$ by two-tailed independent t test corrected for Bonferroni inequality. NMDA acetate receptors were decreased significantly more than other receptors ($P < 0.025$, Kruskal-Wallis nonparametric one-way ANOVA).

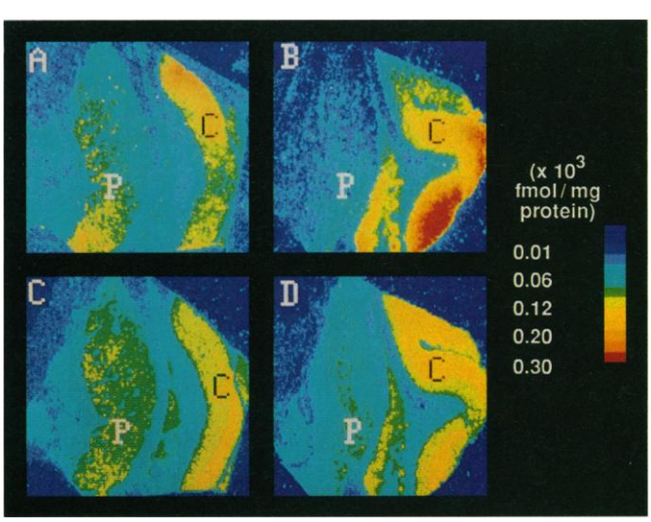

Fig. 2. Quantitative color transform of autoradiographs of NMDA receptors measured with 20 n*M* [^{3}H]glutamate in tris-acetate in sections from coronal blocks through putamen of control (**A**) and HD (**B**) brains. PCP receptors measured in 40 n*M* [^{3}H]TCP were also measured in the same control (**C**) and HD (**D**) brains. Colors represent various amounts of binding in femtomoles per milligram of protein, as shown on the color bar. Abbreviations: C, insular cortex; and P, putamen.

sections were washed for 30 min and then incubated with 20 n*M* [^{3}H]glutamate in 50 m*M* tris-acetate buffer, *p*H 7.2, with 2.5 μ*M* quisqualate to block binding to quisqualate receptors, with or without 1 m*M* L-glutamate as a blank (*12, 15*). This assay optimizes binding to NMDA receptors (*15, 16*). [^{3}H]*N*-1-(2-thienyl)cyclohexyl-3,4-piperidine ([^{3}H]TCP) binding to the phencyclidine (PCP) receptor was also assayed (*15*). The PCP receptor is closely associated with the NMDA receptor channel (*17*). Sections were incubated in 40 n*M* [^{3}H]TCP and 50 m*M* tris-acetate buffer plus 1 m*M* Mg acetate, *p*H 7.2, with or without 10 μ*M* TCP as a blank. Sections received three 5-min rinses in cold buffer and were then processed as described above.

[^{3}H]Glutamate binding to NMDA receptors in tris-acetate in the presence of quisqualate was decreased by 93% in HD putamen and [^{3}H]TCP binding was decreased by 67% (Figs. 1 and 2). In this second group of brains, [^{3}H]glutamate binding measured in tris-HCl–$CaCl_2$ buffer showed the same results as the first group; therefore results from both sets of HD and control brains for tris-HCl–$CaCl_2$ buffer were combined (Fig. 1). In the second group of brains, no statistically significant differences in cortical NMDA or PCP receptors were observed, although the mean of the HD brains was higher than the mean of the controls.

The changes in NMDA receptors in HD putamen measured in tris-acetate buffer were greater than the changes in quisqualate, GABA, benzodiazepine, and muscarinic cholinergic receptors in the same region ($P < 0.025$ by Kruskal-Wallis nonparametric one-way ANOVA). The NMDA receptors were completely lost in some cases. This receptor change is unlikely to reflect a technical artifact because it was observed with two different assays. The dramatic loss of these receptors suggests that cells containing NMDA receptors are differentially lost in HD putamen, whereas the other receptors are present on both spared and affected neurons. The failure of PCP-receptor reductions to be as marked as NMDA-receptor reductions is interesting, but the cause is unclear. Studies in additional cases will be necessary to clarify this phenomenon.

In HD, medium-sized spiny striatal neurons containing GABA and enkephalin project to the lateral pallidum and are lost very early in HD, as are neurons containing GABA and substance P that project to the substantia nigra pars reticulata (*18, 19*). GABA- and substance P–containing striatal neurons projecting to substantia nigra pars compacta and to medial pallidum are affected later in the disease (*19*). Somatostatin and acetylcholine interneurons are probably relatively spared in the disease (*6*). One would predict, therefore, that if excitotoxic mechanisms play a role in the pathogenesis of HD, then striatal neurons should be differentially sensitive to the neurotoxins. Animal studies, in fact, have shown that NMDA agonists destroy GABA- and substance P–containing neurons and spare somatostatin and acetylcholine neurons (*7, 9*).

The large decreases observed in striatal NMDA receptors in HD striatum could be due to several different mechanisms including overproduction of an endogenous neurotoxin such as quinolinic acid (*10, 20*) or a primary genetic defect in striatal NMDA receptors. Alternatively, HD may produce another defect such as impaired energy metabolism in a subset of striatal neurons. Cells with an abnormal ability to maintain adequate energy stores may be more vulnerable to excitotoxic damage (*21*). If so, then neurotoxic mechanisms, although secondary, might accelerate the course of the disorder. If any of the above mechanisms do play a role in HD, then blockade of NMDA receptors could retard the progression of the disease.

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11. Two groups of brains were used in these experiments. In the first group the average age and postmortem delay of the seven HD cases were 49 ± 7 (SEM) years and 15 ± 6 (SEM) hours compared to 55 ± 6 years and 18 ± 6 hours in the six control cases. The average age and postmortem delay of the second group were 40 ± 10 years and 12 ± 3 hours for the five HD cases and 67 ± 3 years and 19 ± 6 hours for the six controls. Two cases were juvenile onset. The HD cases ranged from stage II to IV pathologically (*3*). Patients took various medications but no consistent drug effects were present to explain the results, and no patient was taking medication known to affect NMDA receptors. In the second group of brains, there was no statistically significant correlation of NMDA binding in tris-acetate buffer in control putamen compared to age ($r = -0.778$, $P < 0.1$), nor of NMDA binding in tris-HCl–$CaCl_2$ in control putamen compared to age ($r = -0.146$). There were no statistically significant correlations with postmortem delay for any group.
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14. In rat brain, 2.5 μM quisqualate and 100 μM NMDA together compete for approximately 95% of total [^{3}H]glutamate binding in 50 mM tris-HCl with 2.5 μM $CaCl_2$. In humans and other primates, however, approximately 20 to 50% of Ca^{2+}- and Cl^--dependent [^{3}H]glutamate binding is not sensitive to quisqualate, kainate, NMDA, 2-amino-4-phosphono-butyric acid, aspartate, or dihydrokainate. This nonquisqualate-, non-NMDA–sensitive site is decreased about 50% in HD putamen compared to control. The significance of this latter binding site is unknown, but the magnitude of its loss is similar to that of the other non-NMDA receptors, suggesting that it is present on both affected and spared neurons. The quisqualate-sensitive [^{3}H]glutamate binding sites measured in this assay are not likely to represent uptake sites, but rather non-NMDA receptors (*12*).
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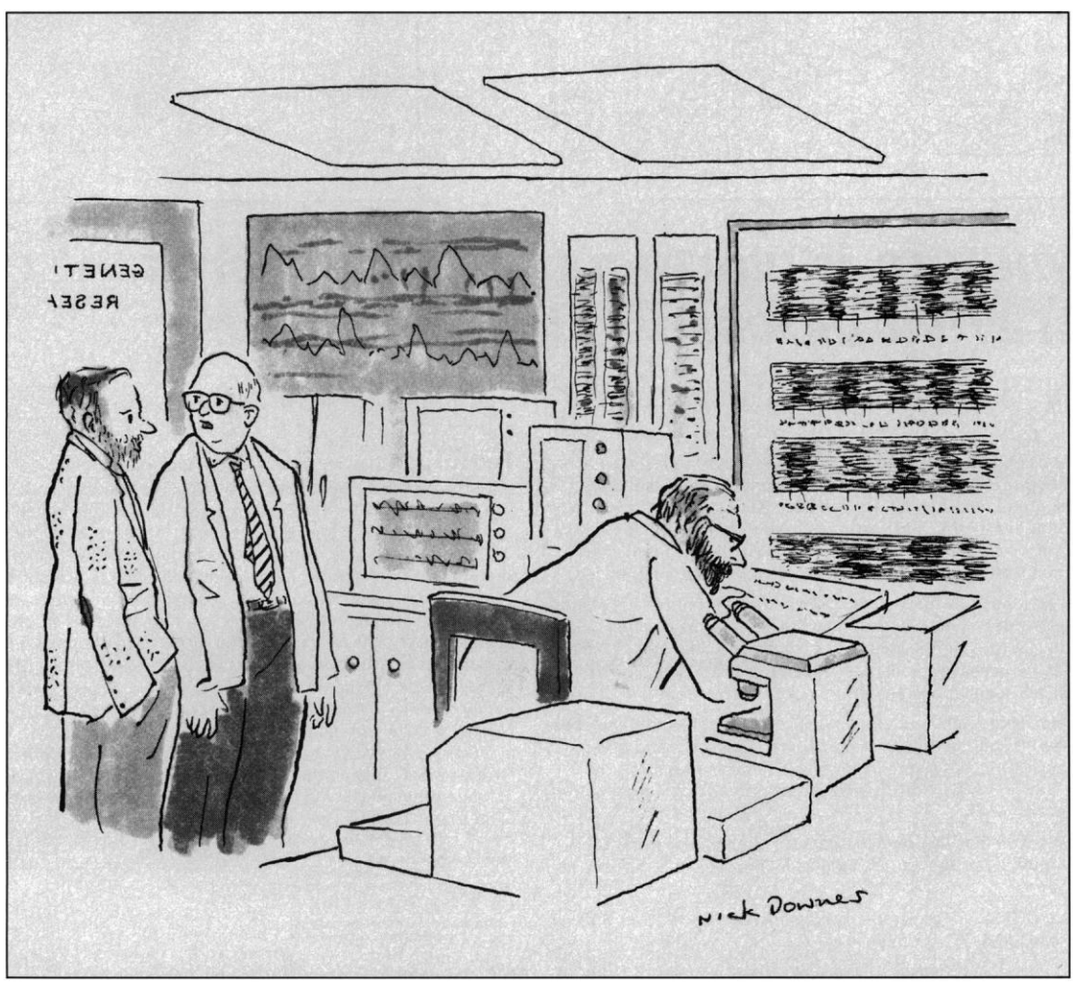

"Dr. Farnsworth is attempting to isolate the gene that makes people do this sort of thing for a living."