Arbacia punctulata sperm take up exogenous [^{32}P]phosphate and incorporate it predominantly into the 160-kD guanylate cyclase, in the form of [^{32}P]phosphoserine (*19*). Within the first 3 seconds after exposure of sperm to egg jelly all label is removed from the cyclase, and the mobility shift to 150 kD is observed (*19*). A similar mobility shift and loss of label from the 160-kD form of the enzyme can be induced in vitro with exogenous phosphatase preparations (*19*). Although partial proteolysis of the 160-kD form of the enzyme cannot be conclusively ruled out, we have suggested on the basis of these observations that the 160- to 150-kD mobility shift seen in vivo may be the result of a jelly-induced dephosphorylation of the 160-kD form of the enzyme. Such a large effect of phosphorylation state on electrophoretic mobility is not without precedent (*21, 22*) and is presumably due to an effect of charged phosphate groups on sodium dodecyl sulfate binding (*23, 24*). Egg jelly is known to induce an increase in protein phosphatase activity in sea urchin sperm (*16*).

In summary, we have demonstrated that the guanylate cyclase of sea urchin sperm is a phosphoprotein, that extracellular factors from the egg induce a change in its electrophoretic mobility (possibly as a result of dephosphorylation), and that correlated with this change in electrophoretic mobility is a change in guanylate cyclase activity. These results may help to elucidate the mechanisms by which extracellular factors from the egg activate the spermatozoan during fertilization.

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References and Notes

1. D. L. Garbers and G. S. Kopf, *Adv. Cyclic Nucleotide Res.* **13**, 251 (1980).
2. G. K. SeGall and W. J. Lennarz, *Dev. Biol.* **71**, 33 (1979).
3. H. Ohtake, *J. Exp. Zool.* **198**, 303 (1976).
4. G. S. Kopf, D. J. Tubb, D. L. Garbers, *J. Biol. Chem.* **254**, 8554 (1979).
5. G. H. A. Clowes and E. Bachman, *Proc. Soc. Exp. Biol. Med.* **18**, 120 (1921).
6. J. R. Hansbrough and D. L. Garbers, *J. Biol. Chem.* **256**, 2235 (1981).
7. R. W. Schackmann, E. M. Eddy, B. M. Shapiro, *Dev. Biol.* **65**, 483 (1978).
8. R. W. Schackmann and B. M. Shapiro, *ibid.* **81**, 145 (1981).
9. F. Collins and D. Epel, *Exp. Cell Res.* **106**, 211 (1977).
10. D. R. Repaske and D. L. Garbers, *J. Biol. Chem.* **258**, 6025 (1983).
11. R. W. Schackmann, R. Christen, B. M. Shapiro, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6066 (1981).
12. D. L. Garbers, H. D. Watkins, J. R. Hansbrough, A. Smith, K. S. Misono, *J. Biol. Chem.* **257**, 2734 (1982).
13. A. E. Levine, K. A. Walsh, E. J. B. Fodor, *Dev. Biol.* **63**, 299 (1978).
14. Y. Yamada, T. Matsui, K. Aketa, *Eur. J. Biochem.* **122**, 57 (1982).
15. D. L. Garbers, D. J. Tubb, G. S. Kopf, *Biol. Reprod.* **22**, 526 (1980).
16. G. Swarup and D. L. Garbers, *ibid.* **26**, 953 (1982).
17. A. F. Conway and C. B. Metz, *J. Exp. Zool.* **198**, 39 (1976).
18. G. K. SeGall and W. J. Lennarz, *Dev. Biol.* **86**, 87 (1981).
19. G. E. Ward and V. D. Vacquier, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 5578 (1983).
20. E. W. Radany, R. Gerzer, D. L. Garbers, *J. Biol. Chem.* **258**, 8346 (1983).
21. R. E. Gates and L. E. King, *Biochem. Biophys. Res. Commun.* **105**, 57 (1982).
22. A. D. Wegenar and L. R. Jones, *J. Biol. Chem.* **259**, 1834 (1984).
23. A. K. Dunker and R. R. Rueckert, *ibid.* **244**, 5074 (1969).
24. R. T. Swank and K. D. Munkres, *Anal. Biochem.* **39**, 462 (1971).
25. G. Gillis, R. Peterson, L. Russell, L. Hook, M. Freund, *Prep. Biochem.* **8**, 363 (1978).
26. S. Podell and V. D. Vacquier, *Exp. Cell Res.*, in press.
27. M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, F. Smith, *Anal. Chem.* **28**, 350 (1956).
28. Concanavalin A (Sigma), 200 mg, was dissolved in 25 ml of 100 m*M* Hepes (*p*H 7.5), 10 m*M* $CaCl_2$, 5 m*M* $MnCl_2$, and 100 m*M* α-methyl-D-mannoside. Undissolved material was removed by centrifugation (15 minutes at 15,000*g*). Affigel (Bio-Rad; 25 ml) was washed (2°C) with 3 volumes of isopropanol and 3 volumes of H_2O and immediately added to the concanavalin A solution (cooled to 2°C). The slurry was stirred gently on ice for 4 hours. The coupled resin was washed batchwise in 100 ml of buffer B [10 m*M* Hepes (*p*H 7.5), 150 m*M* NaCl, and 10 m*M* $CaCl_2$] supplemented with 100 m*M* α-methyl-D-mannoside, then suspended in 25 ml of buffer B, 100 m*M* α-methyl-D-mannoside, and 0.5*M* glycine, and gently stirred for 1 hour at 21°C. The resin was then washed with 100 ml of buffer B followed by 100 ml of 0.25*M* $NaHCO_3$ (*p*H 8.8), suspended in 100 ml of 0.25*M* $NaHCO_3$ (*p*H 8.8), 0.05 percent (by volume) glutaraldehyde, stirred gently for 1.5 hours at 21°C, and washed with 100 ml of 0.25*M* $NaHCO_3$ (*p*H 8.8). Finally, the resin was suspended in 100 ml of 1*M* tris (*p*H 7.8), stirred gently for 1 hour at 21°C, washed extensively with buffer B, and stored at 4°C in buffer B with 0.05 percent (weight to volume) Na_3N. Stored resin was washed (21°C) with 100 volumes of buffer A supplemented with 300 m*M* α-methyl-D-mannoside, 1.25*M* NaCl, 10 percent (by volume) ethylene glycol, and then 750 volumes of buffer A (2°C) prior to use.
29. D. L. Garbers and F. Murad, *Adv. Cyclic Nucleotide Res.* **10**, 57 (1979). Final assay conditions (21°C) were: 0.125 percent Triton X-100, 71 m*M* NaCl, 2.5 m*M* $CaCl_2$, 3 m*M* $MnCl_2$, 8 m*M* Na_3N, 10 m*M* NaF, 200 μ*M* sodium vanadate, 3 m*M* dithiothreitol, 2.5 m*M* benzamidine-HCl, 1.8 percent glycerol, 5.5 m*M* α-methyl-D-mannoside, 1 m*M* 3-isobutyl-1-methylxanthine, 1 m*M* guanosine 3′,5′-monophosphate (cyclic GMP), 0.5 m*M* guanosine triphosphate (GTP) [including [^{3}H]GTP (ICN) at 10^6 cpm per 200-μl assay tube], 30 m*M* tris, 5 m*M* *N*-morpholinoethanesulfonic acid (MES) (*p*H 6.5). Samples were diluted so that product formation was linear with protein concentration and time. Whole sperm protein was prepared for the assay by washing jelly-treated sperm through cavitation buffer (centrifuged for 12 minutes at 4000*g*), resuspension in buffer C (2°C), and nitrogen cavitation (2°C) at 400 psi for 10 minutes.
30. S. Udenfriend *et al.*, *Science* **178**, 871 (1972).
31. J. H. Morrissey, *Anal. Biochem.* **117**, 307 (1981).
32. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
33. We thank G. S. Kopf and G. Schatten for helpful suggestions, and E. W. Radany for his gift of antiserum. Supported by NIH grants HD-10254 (to D.L.G.) and HD-12986 (to V.D.V.).
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Evidence for Degenerative and Regenerative Changes in Neostriatal Spiny Neurons in Huntington's Disease

Abstract. *Golgi impregnations of neostriatum from deceased Huntington's disease patients and controls were examined. In all cases of Huntington's disease the morphology of dendrites of medium-sized spiny neurons was markedly altered by the appearance of recurved endings and appendages, a decrease or increase in the density of spines, and abnormalities in the size and shape of spines. Pathological changes were rarely observed in medium-sized and large aspiny neostriatal neurons. The findings provide evidence for simultaneous degeneration and growth of spiny neurons in Huntington's disease and support the view that a specific population of neostriatal neurons is selectively involved in its pathogenesis.*

Huntington's disease (HD) is a genetic disorder characterized in its late stages by severe motor and intellectual impairment (*1*). The cause of the disease is unknown. In neuropathologic studies of HD neuronal loss has been observed in many brain areas, including the basal ganglia and cerebral cortex (*2*). The caudate nucleus is thought to be the primary region affected because it consistently exhibits the most marked atrophy and cell loss. Studies have suggested that there may be a proportionally greater loss of small to medium-sized neurons than large cells in the neostriatum (*3*).

Golgi impregnations of the neostriatum in many species, including the human, reveal at least four types of neurons of small to medium size (*4*). The cell type impregnated most frequently is the medium-sized (diameter, 15 to 20 μm) spiny neuron, which has numerous dendritic spines and a long axon. It has been the best characterized of all neostriatal cell types in anatomical, immunohistochemical, and physiological studies (*5*).

In addition to being used in the study of normal cytoarchitecture, the Golgi method has been used in many brain

areas to examine neuropathologic changes associated with a variety of human disorders. These include unclassified mental retardation (*6*), ganglioside storage diseases (*7*), and Alzheimer's disease (*8*). In the study reported here we used the Golgi method to examine the neostriatum in brains of deceased HD patients. We found marked morphological changes in the dendrites of medium-sized spiny neurons.

Neostriatal tissue from ten HD and nine control subjects was examined. For all HD patients (41 to 73 years old), clinical histories and neuropathologic reports confirming the diagnosis were available. The controls included age-matched normals (n = 4; 48 to 71 years old) and a group with other neurological disorders (n = 5), including Wilson's disease (one patient 23 years old) and Parkinson's disease (one patient 80 years old). These two disorders affect primarily the basal ganglia (*9*). The other controls consisted of a patient (79 years old) who had survived for 9 months after a massive cortical infarct and two other patients (77 and 80 years old) clinically diagnosed as schizophrenics.

Brains were obtained between 1 and 10 hours after death (except for one normal control brain taken at 24 hours) (*10*) and were placed in 10 percent buffered Formalin for 2 to 35 days. Coronal slices were made and blocks approximately 1.5 cm^2 by 4 mm thick were dissected from comparable areas of the caudate and putamen at the level of the anterior commissure and globus pallidus. In one HD subject (72 years old) only the most rostral portion of the caudate nucleus was available for study. All tissue was processed by the rapid Golgi procedure (*11*).

The density of cellular impregnation varied from case to case. In general, more impregnated neurons appeared in control than HD group samples, and in the latter more impregnated cells were present in the putamen than in the caudate. Spiny neurons were always more prevalent than any other type of cell. In caudate nuclei from HD patients most medium-sized neurons were severely degenerated and could not be classified. These cells were characterized by a few thin, truncated dendrites with irregular contours and few or no spines. The cell bodies, initial segments of axons, and primary dendrites were also irregular in contour, with some focal swellings. Other neurons in the caudate and putamen were less atrophic and retained the dendritic branching and spine distribution characteristic of medium-sized spiny cells (*12*). However, two features of spiny neurons, the orientation of dendrites and the density of spines, were markedly altered.

Normally, terminal segments of spiny neuron dendrites are long, course radially outward, and end after briefly tapering in diameter (Figs. 1a and 2a). In the HD tissue, however, one or more terminal segments of these dendrites recurved abruptly and coursed circuitously for various distances (50 to 150 μm) or turned inward toward the soma (Figs. 1b and 2, b to f). More than one bend occurred in some segments, resulting in s-shaped, spiral, or figure-eight patterns (Fig. 2, b to e). Some curved segments branched anomalously into shorter segments before terminating (Fig. 2d). Also, terminal segments belonging to one or more neurons recurved into the same regions of neuropil and appeared to intertwine (Fig. 2e).

Recurved dendrites were abundantly evident in all tissue samples from HD patients examined. A quantitative study of six of the samples (*13*) showed that 63 to 100 percent of neostriatal spiny neurons had one or more recurved dendritic tips (Fig. 4a). Only the caudate nucleus taken from the most rostral level of the neostriatum showed proportionally fewer affected neurons (34 percent). The

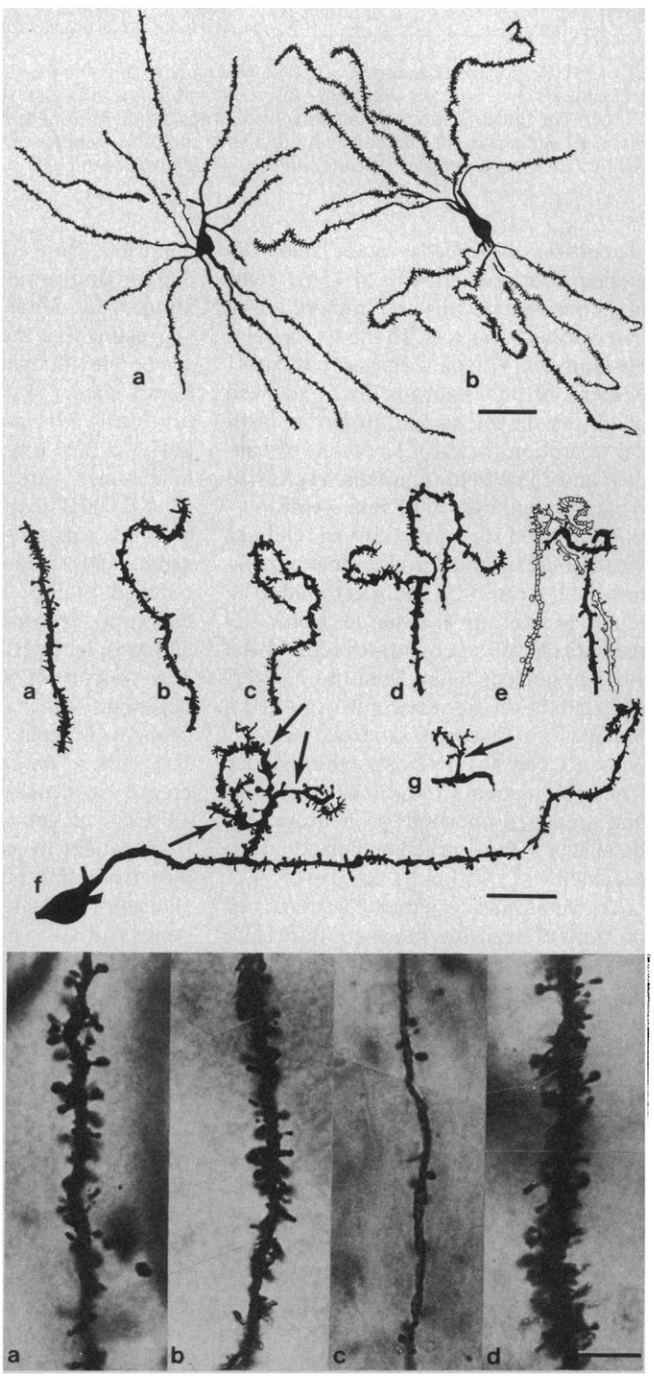

Fig. 1 (top). Camera lucida drawings of neostriatal spiny neurons. (a) Cell from a normal control, showing typical radiating dendrites. (b) Neuron from an HD patient, showing marked recurving of terminal dendritic branches. Scale bar, 50 μm. Fig. 2 (middle). Spiny dendrites from normal neurons (a) and from neurons from HD patients (b to g). Dendrites in diseased neurons recurve in a variety of ways (b to d) and may become tightly intertwined with each other (e). Abnormal dendritic growth may occur in the form of new collateral branches (arrows in e) that have long thin spines and large bulbous spines. The portion of dendrite shown in (g) issues an abnormal dendritic appendage (arrow). Scale bar, 25 μm. Fig. 3 (bottom). Spiny dendrites from normal neostriatum (a) and from neostriatum from HD patients (b to d). Compared to normals (a), the density of spines in HD neurons may be unchanged (b), severely reduced (c), or markedly increased (d). Scale bar, 10 μm.

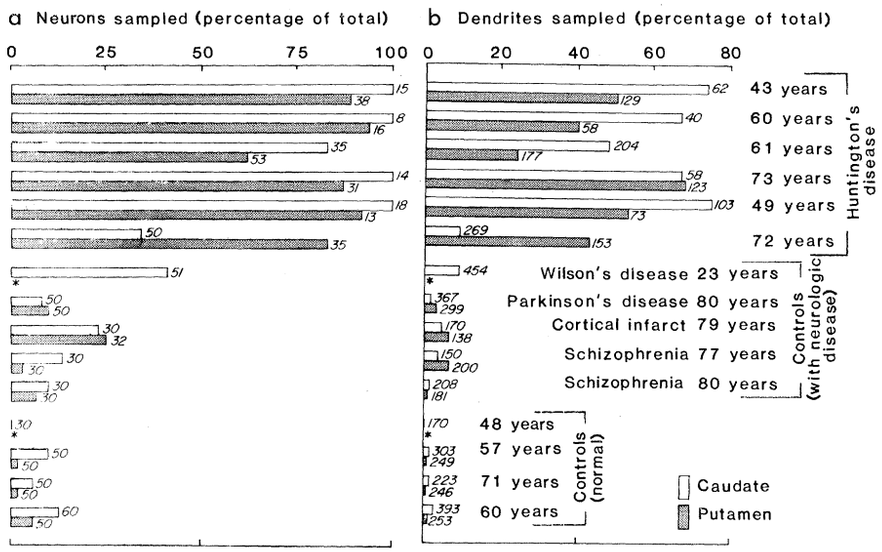

Fig. 4 (a). Proportion of neurons with recurved endings found in the caudate and putamen of six HD patients, five controls with other diseases, and four normal controls. Numbers to the right of each bar indicate the total number of neurons sampled. (b) Proportion of dendrites with recurved endings in the caudate and putamen in the same samples of neurons. Numbers to the right of each bar represent the total number of dendrites sampled.

proportion of neurons with recurved endings was negligible (0 to 13 percent) in all normal controls and in three of the five diseased controls. In the tissue sample from the Wilson's disease patient 41 percent of the neurons had recurved dendrites. In the sample from the cortical infarction patient 23 percent of caudate and 25 percent of putamen cells had recurved dendrites. The relative proportion of dendrites with curved endings was also determined in the same populations of neurons (*13*) and was found to be much greater in the tissue from HD patients (23 to 75 percent) (except for the caudate nucleus taken from the 72-year-old) than in the normal controls (0 to 2.5 percent) or diseased controls (1 to 9 percent) (Fig. 4b). In most of the samples from HD patients the caudate nucleus had greater proportions of neurons and dendrites with recurved endings than the putamen.

To determine whether the recurved portions of dendrites in neostriatum from HD patients represented new growth, we measured curved and uncurved dendrites in the HD group and straight dendrites in the normal controls (*14*). The mean length of curved dendrites in the HD group (283.7 ± 39.6 μm; $n = 31$ dendrites) was greater than that of straight dendrites in the normal controls (265 ± 19.1 μm; $n = 20$), but not significantly so. The standard deviations, however, were significantly different ($P < 0.001$, Bartlett test), reflecting the greater range of dendritic lengths among the spiny cells of HD patients. More important, nearly one-third of the curved dendrites from HD patients exceeded 300 μm and were longer than any of the straight dendrites of normal controls, suggesting that the curved dendrites had grown. In the same cells from HD patients, uncurved dendrites (220.7 ± 48.2 μm; $n = 29$) and curved dendrites (207.7 ± 32.7 μm from origin to point of inflection) were significantly shorter ($P < 0.0001$) than straight dendrites of controls, suggesting that the overall dendritic field radius of spiny neurons is reduced in HD. These results suggest that spiny neurons undergo regeneration and atrophy in HD.

A concurrent process of growth and degeneration may also explain the changes in spine density observed in HD, since marked increases and decreases in density were observed (Fig. 3). Loss of spines varied considerably from patient to patient. Of the six samples from HD patients examined, two showed relatively little change in the density of spines compared to normals, whereas in the other four 20 to 80 percent of the spiny cells showed a marked depletion of spines (*15*). Severe loss of spines was usually associated with a reduction in dendritic diameter (Fig. 3c). Spine loss was not specific to the neostriatum of HD patients, since neurons with a low density of spines were noted in the 80-year-old schizophrenic (about 50 percent of spiny neurons were affected) and in the cortical infarct patient (about 80 percent). Spine density in a smaller proportion of HD neurons (6 to 14 percent) was abnormally increased by as much as 50 percent (*15*). Changes in spine density in a cell were either focal, affecting only small segments (10 to 20 μm) of a dendrite, or more uniform in distribution, and they occurred in both the curved and uncurved dendrites.

In addition to recurved dendrites and alterations in spine density, some neurons (about 10 percent) exhibited abnormally long, thin spines and large bulbous ones, and some had larger appendages that resembled newly formed dendrites (Fig. 2, f and g).

Neurons with degenerative changes (as evidenced by a decrease in spine density) were relatively more numerous in the caudate than in the putamen, whereas neurons with normal-appearing or regenerating dendrites marked by the presence of hyperspinous patches, large spines, and appendages were more prevalent in the putamen (*16*). These regional differences are in accord with the well-recognized topographic gradient of cell loss encountered in HD (*3*).

Other types of neurons in our samples from HD patients consisted mostly of medium- and large-sized aspiny neurons. Aspiny neurons were not visibly different from those of normal controls, except for occasional swellings in the proximal and distal dendrites of the large neurons.

The observed changes in the dendritic morphology of neostriatal spiny neurons in HD are consistent with the impression, gained from routine cell stains, that medium-sized neurons may be more affected in HD than larger neurons (*3*). Our results are also in agreement with a reported decrease in the brains of HD patients in the content of γ-aminobutyric acid, substance P, and enkephalins—putative neurotransmitters that occur primarily in medium-sized spiny neurons (*17, 18*).

The alterations in medium-sized spiny neurons in HD appear to occur in two sequential but overlapping phases. An earlier reactive state involves the growth of dendrites (long recurved endings, appendages, and increased density and size of spines). As the disease progresses, degenerative events characterized by a loss of spines and dendritic segments predominate. This concurrence of regenerative and degenerative changes in spiny cells in HD is analogous to the cytopathology of cortical cells described in some cases of Alzheimer's disease (*8*).

Recurving of terminal dendrites in neostriatal medium-sized spiny neurons appears to be highly characteristic of HD (*19*). Furthermore, the high frequency of recurved dendrites may be a clue to the pathophysiology of the disease. Evidence for dendritic growth at recurved

endings, along with observed increases in spine density and the appearance of newly formed dendritic branches, suggests that neurons are responding to abnormal cues from the internal or external milieu of the cell. A metabolic disturbance in the neuron, perhaps under the control of the HD gene, may lead to dendritic hyperplasia. Such a condition is thought to account for the appearance of "meganeurites" in cortical pyramidal cells in variants of Tay-Sachs disease in which there is an abnormal accumulation of gangliosides (*7*). Alternatively, neurons in HD may be influenced by trophic factors in the local environment because of the loss of intrastriatal or extrinsic afferent inputs. Studies in rats have shown that reorientation and growth of dendrites and spines can occur after denervation of the dentate gyrus (*20*).

The loss of spines in HD is a more variable feature than the recurving of dendrites and may be secondary to other pathological events in the brain. Spine loss in the neostriatum in HD may be dependent on the degree of atrophy or cell loss in the cerebral cortex (*2*). Cortical neurons provide a major source of afferents to the neostriatum, where they synapse primarily with dendritic spines (*21*), and experiments have shown that the density of spines in neostriatal neurons is markedly reduced after cortical deafferentation (*22*). Moreover, a marked loss of spines in neostriatal spiny neurons was observed in one of our diseased controls in which there was extensive damage to the cortex.

In summary, Golgi impregnations of the neostriatum provide morphological evidence that a specific type of neostriatal neuron, the spiny cell, may be selectively altered in HD. Our findings suggest that the dendrites of spiny neurons exhibit a variety of changes in HD which have regenerative and degenerative characteristics. The relative frequency of affected neurons and the types of changes observed can be correlated with their location in the neostriatum.

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References and Notes

1. G. W. Paulson, *Adv. Neurol.* **23**, 177 (1979).
2. G. W. Bruyn, G. T. A. M. Bots, R. Dom, *ibid.*, p. 83.
3. R. Dom, F. Baro, J. M. Bruchner, in *Huntington's Chorea: 1872–1972*, A Barbeau, T. N. Chase, G. W. Paulson, Eds. (Raven, New York, 1973), p. 369; H. Lange, G. Thorner, A. Hopf, K. F. Schroeder, *J. Neurol. Sci.* **28**, 401 (1976).
4. H. Braak and E. Braak, *Cell Tissue Res.* **227**, 319 (1982); H. T. Chang, C. Y. Wilson, S. T. Kitai, *J. Comp. Neurol.* **298**, 197 (1982) M. DiFiglia, P. Pasik, T. Pasik, *Brain Res.* **114**, 245 (1976); M. Eder, T. Vizkelety, T. Tömbol, *Acta Morphol. Acad. Sci. Hung.* **28**, 337 (1980); C. A. Fox, A. N. Andrade, D. E. Hillman, R. C. Schwyn, *J. Hirnforsch.* **13**, 181 (1971/1972); G. A. Graveland, R. S. Williams, M. DiFiglia, *J. Comp. Neurol.*, in press; J. M. Kemp and T. P. S. Powell, *Philos. Trans. R. Soc. London Ser. B* **262**, 383 (1971).
5. M. DiFiglia, P. Pasik, T. Pasik, *J. Neurocytol.* **9**, 471 (1980); M. DiFiglia, N. Aronin, J. B. Martin, *J. Neurosci.* **2**, 303 (1982); R. J. Preston, G. A. Bishop, S. T. Kitai, *Brain Res.* **183**, 253 (1980); P. Somogyi, J. P. Bolam, A. D. Smith, *J. Comp. Neurol.* **195**, 567 (1981); C. J. Wilson and P. M. Groves, *ibid.* **194**, 599 (1980).
6. P. R. Huttenlocher, *Neurology* **24**, 203 (1974); R. S. Williams, S. L. Hauser, D. P. Purpura, G. R. DeLong, C. H. Swisher, *Arch. Neurol.* **37**, 749 (1980).
7. D. P. Purpura, in *Congenital and Acquired Cognitive Disorders*, R. Katzman, Ed. (Raven, New York, 1979), p. 43.
8. A. Scheibel, *ibid.*, p. 107.
9. Wilson's disease is a metabolic storage disease in which the globus pallidus and putamen are atrophic; Parkinson's disease involves a disturbance in the nigrostriatal dopaminergic pathway.
10. Impregnation artifacts due to long postmortem delays, as previously described in rats [R. S. Williams, R. J. Ferrante, V. S. Caviness, Jr., *J. Neuropathol. Exp. Neurol.* **37**, 13 (1978)], were not present in any of the tissues examined in this study.
11. Blocks were placed in an osmium dichromate solution for 2 to 5 days, transferred to 0.75 percent silver nitrate for another 2 to 3 days, and embedded in celloidin. Sections (100 μm) were cut and mounted in serial order.
12. In normal brains spiny neurons exhibit up to a 5th-order branching, which occurs in the proximal half of the dendritic field. Proximal dendrites are typically spine-free, whereas distal branches are covered with numerous spines.
13. Six of the ten HD group samples were selected for quantitative study because they contained sufficient numbers of impregnated neurons in the caudate and putamen. Slides from the HD and control groups were coded, randomized, and examined under the light microscope at ×400. Sections were scanned systematically and neurons coming into the field of view were evaluated. For each cell, all dendrites that could be followed to their termination were recorded. A bend in the dendrite of 90° or more was established a priori as the criterion for a recurved ending. Results were expressed as the percentage of neurons with recurved dendrites (Fig. 4a) and the percentage of dendrite endings with recurved tips (Fig. 4b).
14. Dendrites (defined here as a stem segment and all its subordinate branches) from the HD and normal control groups were examined for overall length with a computer-assisted light microscope. Dendrites selected for measurement (60 HD group and 29 control dendrites) were optimally oriented in the plane of tissue section. A calibrated eyepiece reticle and a ×100 oil immersion lens were used. Starting at the dendrite origin, reference points marking 10-μm segments were entered into the computer [R. S. Williams and S. Matthysse, *J. Comp. Neurol.* **215**, 154 (1983)] and the overall length of the dendrite was determined by adding the segments.
15. Neurons studied for recurved endings (*13*) were also examined qualitatively for spine density. A neuron was rated as "low" if the density of spines in most dendrites appeared to be less than half that typical of most spiny neurons in normal controls or "high" if spine density in one or more dendrites exceeded that typical of normals. To check the validity of the ratings, spines were counted in some dendrites from cell body origin to termination with a calibrated eyepiece reticle and a ×100 oil immersion lens. The density of spines in normal controls (n = 10 dendrites) ranged from six to eight spines per 10 μm of dendrite. In HD spiny neurons, spine density ranged from three to four spines per 10 μm (n = 10 dendrites) for neurons with a low spine density rating and 12 to 15 spines per 10 μm (n = 10 dendrites) for neurons with a high density rating.
16. It should be emphasized that individual HD neurons could exhibit both atrophic and regenerative changes. For example, a neuron with marked spine loss might also have abnormally long recurved dendrites or dendritic appendages.
17. J. P. Bolam, P. Somogyi, H. Takagi, I. Fodor, A. D. Smith, *J. Neurocytol.* **12**, 325 (1983); M. DiFiglia, N. Aronin, J. B. Martin, *J. Neurosci.* **2**, 303 (1982); C. E. Ribak, J. E. Vaughn, E. Roberts, *J. Comp. Neurol.* **187**, 261 (1979).
18. P. C. Emson, A. Arregui, V. Clement-Jones, B. E. B. Sandberg, M. Rossor, *Brain Res.* **199**, 147 (1980); T. L. Perry, S. Hansen, M. Kloster, *N. Engl. J. Med.* **288**, 337 (1973).
19. On the basis of our clinical records, this is unlikely to be a pharmacological effect. Of the six HD patients studied quantitatively, two were on haloperidol, one was on Benadryl, and two were not taking any medication. No information on the other patient was available. One of the two psychotic patients (77 years old) was on phenothiazines.
20. J. G. Parnavelas, G. Lynch, N. Brecha, W. W. Cotman, A. Globus, *Nature (London)* **248**, 71 (1974); A. Cacereo and O. Steward, *J. Comp. Neurol.* **214**, 387 (1983).
21. R. Hassler, J. W. Chung, U. Rinne, A. Wagner, *Exp. Brain Res.* **31**, 67 (1978); J. M. Kemp and T. P. S. Powell, *Philos. Trans. R. Soc. Ser. B* **262**, 413 (1971).
22. J. M. Kemp and T. P. S. Powell, *Philos. Trans. R. Soc. Ser. B* **262**, 429 (1971).
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Computer Graphics Representation of Levels of Organization in Tobacco Mosaic Virus Structure

Abstract. *Methods for simplifying computer graphics images of atomic models of complex macromolecular assemblies have been applied to the tobacco mosaic virus structure to display different levels of its organization. By constructing sharply outlined pictures of the parts of the virus particle with the image resolution reduced or with obscuring detail eliminated, aspects of the subunit packing and chain folding are distinctly illustrated.*

Structures of increasingly complex macromolecular assemblies are being solved to the atomic level by x-ray diffraction analysis. Computer graphics methods have been applied to macromolecular structures to produce high-resolution color pictures of the molecular surfaces (*1*) and to display details of surface interactions and the underlying skeletal structure (*2*). Regardless of whether the atomic arrangements are represented by space-filling or by skeletal models, only relatively small portions of complex molecules can be effectively visualized at one time. The foldings of protein backbones into α-helical and β-