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Striatal neuronal loss correlates with clinical motor impairment in Huntington's disease

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Abstract

Background—Huntington disease is characterized clinically by chorea, motor impairment, psychiatric manifestations and dementia. Atrophy of the striatum is the neuropathological hallmark of Huntington disease and previous studies have suggested that striatal atrophy correlates more closely with motor impairment than with chorea. Motor impairment, as measured by motor impairment score, correlates with functional disability in Huntington patients, but chorea does not. In this study, we investigate the relation between neuronal loss and these motor features.

Methods—We conducted neuropathological and stereologic assessments of neurons in putamen and subthalamic nuclei in Huntington patients and age-matched controls. In putamen, we estimated the total number and volume of medium spiny neurons labeled with dopamine- and cAMP-regulated phosphoprotein 32 kDa (DARPP32). In subthalamic nuclei, we estimated the total number of neurons on Hematoxylin/Eosin -Luxol/Fast Blue stains.

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Author's Roles

Zhihong Guo contributed to the conception, organization and execution of the project; statistical analysis, and wrote the first draft of the manuscript and implemented multiple reviews. Gay Rudow contributed to the stereological analyses. Olga Pletnikova contributed to the organization and execution of the project. Barbara Crain, Kari-Elise Codispoti and Brent A. Orr conducted neuropathological autopsies and formulated diagnoses. Wenzhen Duan contributed to the manuscript writing and review. Russell Margolis and Adam Rosenblatt were the clinicians who followed the patients in the Huntington's disease clinic. Christopher A. Ross participated in the clinical assessments, conception and initiation of the project, manuscript writing and review. Juan C. Troncoso contributed to the conception, organization, and execution of the project, and participated in the preparation and review of the manuscript. All authors reviewed the manuscript.

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Results—In putamen of Huntington disease, immunohistochemistry showed DARPP 32 neuronal atrophy with extensive disruption of neurites and neuropil; stereologic studies found significant decreases in both the number and size of DARPP32 neurons; we also detected a significant reduction of overall putamen volume in Huntington patients compared with controls. In subthalamic nuclei, there was a mild but significant neuronal loss in Huntington group. The loss of neurons in putamen and subthalamic nuclei and the putamenal atrophy were significantly correlated with the severity of motor impairment, but not with chorea.

Conclusions—Our findings suggest that neuronal loss and atrophy in striatum and neuronal loss in subthalamic nuclei contribute specifically to the motor impairment of Huntington, but not to chorea.

Keywords

Putamen; subthalamic nucleus; medium spiny neurons; DARPP32; stereology

Introduction

Huntington disease (HD) is caused by an expanded CAG repeat in the huntingtin gene, coding for a long stretch of polyglutamines in the huntingtin (htt) protein^{1–5}. Factor analysis of the two major motor scales used in HD (the quantitative neurological exam and the motor UHDRS) indicate that there are two major components of the movement disorder—chorea and “motor impairment”^{6, 7}. Previous studies suggest that chorea often predominates early in the disease; while motor impairment is more characteristic of advanced HD and is associated with greater pathological severity and length of CAG repeats^{8, 9}.

Neuroanatomical and biochemical studies indicate that motor abnormalities of HD are due to degenerative changes of the striatum, globus pallidus, subthalamic nucleus (STN), and substantia nigra pars reticulata^{10–12}. Previous studies have examined the loss of neurons in these anatomical regions, but few have examined in detail the correlations between the loss of neurons in any of these specific nuclei and motor abnormalities^{13, 14}.

Neostriatal atrophy is the morphologic hallmark of HD and the basis for grading its neuropathological severity at autopsy¹². Nevertheless, the pathology of HD extends beyond the neostriatum to extrastriatal regions^{4, 15}. Despite a wealth of information on the neuropathology of HD, the pathological substrate of either chorea or motor impairment in HD remains unclear. We previously showed that the motor impairment observed in individuals with HD strongly correlated with the severity of striatal atrophy at autopsy measured on the Vonsattel scale¹², whereas chorea showed no significant correlation⁹. These results strongly suggested that motor impairment in HD may be a reflection of neostriatal neuronal degeneration.

To test this hypothesis, we used design-based stereology to estimate the number and size of neurons in the putamen in autopsy cases of HD and age-matched controls. Because it is difficult to distinguish atrophic putamenal neurons from reactive astrocytes histologically, we labeled medium spiny neurons (MSNs) with an antibody against dopamine- and cAMP-regulated phosphoprotein 32 kDa (DARPP32), a marker for MSNs. Animal studies have shown that about 95% of MSNs are DARPP32 positive in striatum¹⁶. Stereological estimates of absolute neuronal numbers in a nucleus require to examine the entire structure. For this reason, we chose to examine the putamen, which is fairly compact, and not the entire striatum since the caudate has a more complex geometry. Moreover, data from the literature indicate that the degeneration of the putamen in HD is comparable to that of the caudate^{4, 17, 18}. In addition, we compared cell numbers in the caudate and putamen in two HD cases and one control (data not shown) and demonstrated that, at least in these cases,

neuronal numbers are as severely reduced in the putamen as in the caudate. Thus, we believe that neuronal counts from the putamen are representative of the degeneration of the striatum as a whole.

Experimental and clinical studies of human and non-human primates indicate that lesions limited to the striatum are not sufficient to cause chorea, while lesions of the STN or its connections may lead to chorea or hemiballismus^{19–22}. Thus, we assessed the total number of neurons in the STN in HD.

Materials and methods

Subjects

The HD autopsy brains available for studies of the putamen included 6 males and 8 females, ages from 35 to 86 years (average 60.3 ± 16.1 years), with duration of disease from 5 to 33 years (average 18.9 ± 8.4 years). The controls included 6 subjects, 4 males and 2 females, ages from 17 to 81 years (average 48.2 ± 24.9 years). The brains for the STN study included 10 HD cases, a subset of the 14 brains used for assessment of putamen, ranging from 35 to 72 years (average 54.5 ± 14.3 years) and 7 age-matched controls, ages from 21–65 years (average 44.7 ± 19.9 years).

HD brains were from patients followed by the Johns Hopkins University (JHU) Research Center Without Walls for Huntington's Disease. Control brains from subjects without history of neurological disease were obtained from the Brain Research Center of the JHU under institutional review board-approved protocols. Demographic and clinical information on the subjects is presented in Tables 1 and 2.

Clinical evaluations

All subjects in the HD group had their diagnosis genetically confirmed and were followed with periodic neurologic and psychiatric examinations and neuropsychological testing. The neurologic examination included a motor impairment (MIS) and chorea scores, and detailed description of clinical evaluation has been previously reported⁹. The time interval between last clinical examination and death ranged from 5 to 147 months with average of 58.6 ± 41.4 months. All clinical protocols used in the study were approved by the JHU Human Participants Ethics Committee and informed consent was obtained from all participants and families.

Neuropathology

Autopsy and brain tissue dissection were performed at JHU Neuropathology. The diagnostic brain tissue blocks were processed and stained as described previously²³ and diagnosis and grading of severity followed Vonsattel¹². Tissue sections of the substantia nigra were stained with H&E and examined to exclude Lewy body disease. All HD cases in our study were free of Lewy bodies, although most showed mild pigment incontinence. One HD case, with the longest CAG repeat size (65) and the youngest age at death (35 years), had moderate neuronal degeneration and gliosis of the substantia nigra.

The whole putamen was dissected from fixed coronal brain slabs and sliced into 1-cm thick tissue blocks for processing and paraffin-embedding. The putamen has well defined dorsal, lateral, and ventral boundaries. The medial boundary was arbitrarily defined as a line bisecting the internal capsule. The entire putamen was serially cut at $50 \mu\text{m}$, and sections for stereology were selected with a random start and systematic interval (Q 60 sections) and immunostained with DARPP32 antibody. The entire STN was dissected in four 3-mm coronal blocks from the subthalamic region and rostral midbrain, and serial sections were

cut at 50 μm ; sections for stereology were selected with a random start and systematic interval (Q 20 sections) and stained with H&E-Luxol/Fast Blue.

Immunohistochemistry

A subset of 10–12 tissue sections encompassing the whole putamen was selected and immunostained with antibody against DARPP32 (Cell signaling, 1:50), using biotinylated goat anti-rabbit as secondary antibody, and developing immunoreactivity with Vectastain ABC peroxidase kit followed by staining with 3,3-diaminobenzidine (DAB). Detailed description of the immunohistochemistry method can be found in supplementary information.

Stereology

The putamen was outlined in each of the 10–12 immunostained sections laterally by the external capsule and medially by the globus pallidus and the internal capsule (Fig 1A&B)¹². We examined a subset of 10–12 slides from the STN stained with H&E-Luxol/Fast Blue, and the boundaries of the STN were outlined according to Hamani *et al*²⁴. Using a Stereo- Investigator system (MBF Bioscience, Williston VT), we estimated neuronal numbers and measured the volumes of neuronal cell bodies and nuclei described in previous studies^{25, 26}. Detailed description of the stereology method can be found in supplementary information.

Statistical analyses

All statistical analyses were performed using SigmaStat 3.1 (*Systat, Software, Inc*). Detailed description of statistical analyses can be found in supplementary information.

Results

Pathological Observations

Morphological changes in DARPP32 neurons in HD patients—DARPP32 immunostaining in controls revealed abundant putaminal neurons which displayed intense perikaryal staining and extensive neuritic arborization; axons showed their typical varicosities, and dendrites studded with abundant spines. By contrast, remaining DARPP32 neurons in HD patients were sparse and displayed dystrophic neurites, loss of dendritic spines, and cell body atrophy. In cases of HD Vonsattel grades III and IV, DARPP32 neurons showed scalloping and irregularities of their cellular membranes, fainter immunostaining intensity, axonal fragmentation, extensive disruption of neurites, and attenuation of the neuropil. Similar changes, but of lesser magnitude were present in cases of HD Vonsattel grade II (Fig. 1C &D).

DARPP32 neuronal loss and overall volume reduction of the putamen in HD—We found significant loss of DARPP32 neurons in the putamen of HD patients across all VS grades: 58% loss in grade II, 73% in grade III, and 76% in grade IV (Fig. 1E). We also observed a significant reduction in the overall volume of the putamen in HDs compared to controls: 42% decrease in grades II, 53% in grade III, and 56% in grade IV (Fig 1F). In the HD group, there was no significant difference of DARPP 32 neuronal number in putamen or the overall volume of the putamen among VS grades II through IV.

Decrease in cell body size but not in nuclear size of DARPP32 neurons in the putamen of HD—The loss of DARPP32 neurons in the putamen of HD patients was accompanied by a 40% ($p<0.01$) reduction in the volume of the remaining neurons (Fig. 1G). However, we found no difference in nuclear size of DARPP32 neurons between HD patients and controls (Fig. 1H). This resulted in sharp differences in the ratio of nuclear

volume to neuronal volume between the two groups: 0.27 ± 0.05 in controls and 0.41 ± 0.07 in HD.

Significant neuronal loss in STN of HD patients correlates with loss of neurons in putamen—We found a small but significant neuronal loss in the STN in HD patients compared to controls (20%, $p < 0.05$) (Fig. 2A); we also detected a trend for reduction in overall STN volume in HD (Fig 2B). In addition, four HD patients (1 VS II and 3 VS III) had significant loss of neurons in the putamen, but not in the STN. This observation suggests that the pathological changes in STN trail the changes in putamen.

Clinical Pathological Correlations

Correlations between DARPP32 neuronal loss and clinical motor manifestations

—We next investigated whether the loss of DARPP32 neurons correlated with motor impairment score (MIS). We found a significant inverse correlation between the number of DARPP32 neurons and MIS, i.e., HD patients who displayed more neuronal loss had a more severe motor impairment and higher MIS, (Fig. 3A). In contrast, there was no correlation between DARPP32 neuronal loss and the severity of chorea (Fig. 3B). There was a trend for direct correlation between DARPP32 neuronal volume and chorea score ($r = 0.527$, $p = 0.053$), indicating the possibility that neurons with larger remaining volume were associated with higher chorea score. There was, however, no correlation between neuronal volumes and MIS ($r = -0.34$, $p = 0.49$). Multiple linear regression analysis showed that only DARPP 32 neuronal loss had a significant power to predict the change of MIS ($p = 0.049$), whereas neuronal atrophy and CAG repeat size failed to show a significant effect on prediction of MIS.

Correlation between atrophy of the putamen and clinical motor impairment

—We found that the overall reduction in volume of the putamen in HD correlated strongly and significantly with MIS ($p = 0.004$) (Fig. 3C). There was a positive trend for correlations between the volume of the putamen and the number ($r = 0.471$, $p = 0.089$, Fig. 3D) and sizes ($r = 0.382$, $p = 0.178$) of DARPP 32 neurons. These results suggest that loss of neurons and their decrease in size contribute, at least in part, to the atrophy of the putamen.

Significant neuronal loss in STN of HD patients correlates with clinical motor impairment

—There was significant neuronal loss (20%, $p < 0.05$) (Fig. 2A) in the STN in HD patients compared to those in controls. Interestingly, we found a significant inverse correlation between neuronal numbers and MIS ($p = 0.032$) (Fig. 3E), but no correlation with chorea (Fig. 3F), suggesting that neuronal loss in STN may contribute to motor impairment but not to chorea in HD. In addition, four HD patients (1 VS II and 3 VS III) had significant loss of neurons in the putamen but not in the STN

Discussion

Our stereological study of post-mortem HD brains reveals substantial neuronal loss in the putamen and mild neuronal loss in STN. The loss of neurons correlates significantly with motor impairment but not with chorea. This observation supports the hypothesis that motor impairment in HD is caused by neuronal loss, and is consistent with the hypothesis that chorea may be a manifestation of neuronal dysfunction rather than of neuronal loss in early stages of HD³.

Recently, MRI studies have reported significant reduction of striatal volume in HD including premanifest and moderate HD brains^{27,28}. The PREDICT-HD and TRACK-HD longitudinal analyses of striatal atrophy demonstrate steady changes from premanifest gene

carrier to severe HD^{29, 30}. Neuroimaging studies in patients with HD have also suggested an early atrophy in striatum and extrastriatal regions³¹. The correlation between motor manifestations and striatal neuronal degeneration in HD, demonstrated by our investigation, therefore provides a novel perspective that complements imaging studies relevant to the pathogenesis of motor dysfunction in HD.

In our study, the reduction in overall volume of the putamen appears strongly correlated with motor impairment ($r = -0.708$, $p = 0.004$), and both the loss and atrophy of DARPP 32 neurons contribute to putaminal atrophy in HD (See results). The pattern of DARPP 32 neuronal loss paralleled the pattern of putaminal atrophy (Fig. 1E & 1F). In addition to our quantitative study, qualitative examination of DARPP32 immunolabeling revealed a spectrum of morphologic abnormalities in the remaining putaminal neurons. These changes were characterized by perikaryal shrinkage and loss of stained dendritic processes in early stages of HD, whereas axonal-dendritic processes were almost completely destroyed in VS III and IV cases (Fig 1C & 1D). The remaining DARPP 32 neurons in later stages of the disease appeared isolated as they lost their connection with their neighboring nerve cells. Together, the massive loss of both DARPP 32 neurons and their neurites could result in disruption of local circuitry within the striatum and also in its efferent connections and thereby lead to motor dysfunction in HD patients. Our observations are consistent with the Golgi cytoarchitectonic study by Graveland *et al*³². Given the better visualization of dendrites and spine structures with Golgi, it could be helpful to combine Golgi impregnation with DARPP32 immunohistochemistry to study MSNs in HD.

The mechanisms underlying chorea are not fully understood, but both the striatum and STN have been implicated. It has been proposed that chorea might reflect a progressive period of neuronal dysfunction before cell death or an imbalance between different neuronal populations such as enkephalin vs substance P^{15, 33–35}. Indeed, our study documents striking and massive degeneration of DARPP 32 neurons in the putamen, and the remaining atrophic DARPP32 neurons with variable cell sizes in the putamen of HD patients may represent neurons in different stages of neuronal degeneration. Interestingly, we observed a strong trend for correlation between larger volume of remaining DARPP32 positive neurons and a higher chorea score ($r = 0.527$, $p = 0.053$), consistent with the notion that chorea is perhaps the manifestation of neurons that are dysfunctional or in the early stages of degeneration.

Studies from both human and non-human primates indicate that chorea can be produced by destruction of neurons in STN^{36, 37}. Depending on their size and location, lesions in STN can induce hypokinetic or hyperkinetic movement disorders (hemiballismus and choreoathetosis)^{38, 39}. There are no previous stereological studies of the STN in HD, but Lange *et al* reported a 25% loss of STN neurons using a non-stereological method⁴⁰. Using stereology, we identified a mild (20%, $p < 0.05$) but significant neuronal loss in STN in HDs that correlated significantly with motor impairment score but not with chorea (Fig. 3F). However, four HD patients (1 VS II and 3 VS III) had significant loss of neurons in the putamen but not in the STN. This observation suggests that the pathological changes in STN trail the changes in putamen. Furthermore, three of these four patients did have marked motor impairment. Thus, striatal atrophy appears sufficient to cause motor impairment even in the absence of neuronal loss in the STN. Our results further indicate that the loss of STN neurons appears in relatively later stage of HD, compared to striatal MSNs, which are more vulnerable in HDs^{3, 4}.

In our current study, we did not evaluate neuronal loss in the globus pallidus or degeneration of subtypes of striatal projection neurons, (enkephalin- and substance P-containing neurons),

and striatal interneurons in HD^{33–35}. Therefore, it may be interesting for future studies to identify the neurochemical nature of the remaining neurons in the putamen.

An important caveat in the interpretation of our results is the long average time interval between the last clinical evaluation and death. In a previous study of 100 postmortem cases, we found no correlation between Vonsattel grades of motor impairment score and interval between last neurological exam and death. We interpret this to indicate that clinical features of HD are fairly stable at end stage of disease, making clinical-pathological correlations feasible even given relatively infrequent exams in the period preceding death⁹. Therefore, in the current study, even though there was a substantial interval between the last neurological examination and autopsy in some cases, we were still able to detect the significant correlation between DARPP 32 neuronal loss and MIS.

In this study in which the 14 HD cases exhibited a Vonsattel neuropathological severity ranging from grade II to grade IV (Table 2), we only observed a trend for inverse correlation between DARPP32 neuronal loss and Vonsattel grades ($r = -0.464$, $p = 0.091$). HD patients who had longer CAG repeats had a tendency to exhibit more severe DARPP32 neuronal loss ($r = -0.283$, $p = 0.37$); but there was no correlation between age of onset (motor symptoms onset) and DARPP32 neuronal loss ($r = 0.012$, $p = 0.973$).

In conclusion, our findings indicate that neuronal loss and atrophy of the putamen correlate significantly with motor impairment but not with chorea in HD. In advanced HD, there is also a modest loss of neurons in the STN, but this loss does not appear as necessary for the development of motor impairment. Our observations support the hypothesis that motor impairment in HD is caused by neuronal loss, and are consistent with the notion that chorea may be a manifestation of neuronal dysfunction rather than of neuronal loss in early stages of HD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. The Huntington's Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell*. 1993; 72(6):971–983. [PubMed: 8458085]
2. Eidelberg D, Surmeier DJ. Brain networks in Huntington disease. *J Clin Invest*. 2011; 121(2):484–492. [PubMed: 21285521]
3. Ross CA, Tabrizi SJ. Huntington's disease: from molecular pathogenesis to clinical treatment. *Lancet Neurol*. 2011; 10(1):83–98. [PubMed: 21163446]
4. Vonsattel JP, DiFiglia M. Huntington disease. *J Neuropathol Exp Neurol*. 1998; 57(5):369–384. [PubMed: 9596408]
5. Walker FO. Huntington's disease. *Lancet*. 2007; 369(9557):218–228. [PubMed: 17240289]

6. Folstein MF, Folstein SE, McHugh PR. Mini-mental state" A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res.* 1975; 12(3):189–198. [PubMed: 1202204]
7. Marder K, Zhao H, Myers RH, et al. Rate of functional decline in Huntington's disease. Huntington Study Group. *Neurology.* 2000; 54(2):452–458. [PubMed: 10668713]
8. Margolis RL, Ross CA. Diagnosis of Huntington disease. *Clin Chem.* 2003; 49(10):1726–1732. [PubMed: 14500613]
9. Rosenblatt A, Abbott MH, Gourley LM, et al. Predictors of neuropathological severity in 100 patients with Huntington's disease. *Ann Neurol.* 2003; 54(4):488–493. [PubMed: 14520661]
10. Albin RL. Selective neurodegeneration in Huntington's disease. *Ann Neurol.* 1995; 38(6):835–836. [PubMed: 8526454]
11. Lange KW, Javoy-Agid F, Agid Y, Jenner P, Marsden CD. Brain muscarinic cholinergic receptors in Huntington's disease. *J Neurol.* 1992; 239(2):103–104. [PubMed: 1532417]
12. Vonsattel JP, Myers RH, Stevens TJ, Ferrante RJ, Bird ED, Richardson EP Jr. Neuropathological classification of Huntington's disease. *J Neuropathol Exp Neurol.* 1985; 44(6):559–577. [PubMed: 2932539]
13. Ferrante RJ, Beal MF, Kowall NW, Richardson EP Jr, Martin JB. Sparing of acetylcholinesterase-containing striatal neurons in Huntington's disease. *Brain Res.* 1987; 411(1):162–166. [PubMed: 2955849]
14. Myers RH, Vonsattel JP, Stevens TJ, et al. Clinical and neuropathologic assessment of severity in Huntington's disease. *Neurology.* 1988; 38(3):341–347. [PubMed: 2964565]
15. DiProspero NA, Chen EY, Charles V, Plomann M, Kordower JH, Tagle DA. Early changes in Huntington's disease patient brains involve alterations in cytoskeletal and synaptic elements. *J Neurocytol.* 2004; 33(5):517–533. [PubMed: 15906159]
16. Ouimet CC, Langley-Gullion KC, Greengard P. Quantitative immunocytochemistry of DARPP-32-expressing neurons in the rat caudatoputamen. *Brain Res.* 1998; 808(1):8–12. [PubMed: 9795103]
17. Bolam JP, Somogyi P, Takagi H, Fodor I, Smith AD. Localization of substance P-like immunoreactivity in neurons and nerve terminals in the neostriatum of the rat: a correlated light and electron microscopic study. *J Neurocytol.* 1983; 12(2):325–344. [PubMed: 6188809]
18. Harris GJ, Codori AM, Lewis RF, Schmidt E, Bedi A, Brandt J. Reduced basal ganglia blood flow and volume in pre-symptomatic, gene-tested persons at-risk for Huntington's disease. *Brain.* 1999; 122 (Pt 9):1667–1678. [PubMed: 10468506]
19. Comella CL, Shannon KM. Hemiballismus. *Curr Treat Options Neurol.* 1999; 1(1):1–5. [PubMed: 11096689]
20. DeLong MR. Primate models of movement disorders of basal ganglia origin. *Trends Neurosci.* 1990; 13(7):281–285. [PubMed: 1695404]
21. Lee MS, Marsden CD. Movement disorders following lesions of the thalamus or subthalamic region. *Mov Disord.* 1994; 9(5):493–507. [PubMed: 7990845]
22. Vidakovic A, Dragasevic N, Kostic VS. Hemiballism: report of 25 cases. *J Neurol Neurosurg Psychiatry.* 1994; 57(8):945–949. [PubMed: 7914529]
23. Rudnicki DD, Pletnikova O, Vonsattel JP, Ross CA, Margolis RL. A comparison of huntington disease and huntington disease-like 2 neuropathology. *J Neuropathol Exp Neurol.* 2008; 67(4):366–374. [PubMed: 18379432]
24. Hamani C, Saint-Cyr JA, Fraser J, Kaplitt M, Lozano AM. The subthalamic nucleus in the context of movement disorders. *Brain.* 2004; 127(Pt 1):4–20. [PubMed: 14607789]
25. Iacono D, Markesbery WR, Gross M, et al. The Nun study: clinically silent AD, neuronal hypertrophy, and linguistic skills in early life. *Neurology.* 2009; 73(9):665–673. [PubMed: 19587326]
26. West MJ, Gundersen HJ. Unbiased stereological estimation of the number of neurons in the human hippocampus. *J Comp Neurol.* 1990; 296(1):1–22. [PubMed: 2358525]
27. van den Bogaard S, Dumas E, van der Grond J, van Buchem M, Roos R. MRI biomarkers in Huntington's disease. *Front Biosci (Elite Ed).* 2012; 4:1910–1925. [PubMed: 22202007]

28. Weir DW, Sturrock A, Leavitt BR. Development of biomarkers for Huntington's disease. *Lancet Neurol.* 2011; 10(6):573–590. [PubMed: 21601164]
29. Aylward EH, Nopoulos PC, Ross CA, et al. Longitudinal change in regional brain volumes in prodromal Huntington disease. *J Neurol Neurosurg Psychiatry.* 2011; 82(4):405–410. [PubMed: 20884680]
30. Tabrizi SJ, Scahill RI, Durr A, et al. Biological and clinical changes in premanifest and early stage Huntington's disease in the TRACK-HD study: the 12-month longitudinal analysis. *Lancet Neurol.* 2011; 10(1):31–42. [PubMed: 21130037]
31. Hobbs NZ, Barnes J, Frost C, et al. Onset and progression of pathologic atrophy in Huntington disease: a longitudinal MR imaging study. *AJNR Am J Neuroradiol.* 2010; 31(6):1036–1041. [PubMed: 20150305]
32. Graveland GA, Williams RS, DiFiglia M. Evidence for degenerative and regenerative changes in neostriatal spiny neurons in Huntington's disease. *Science.* 1985; 227(4688):770–773. [PubMed: 3155875]
33. Augood SJ, Faull RL, Love DR, Emson PC. Reduction in enkephalin and substance P messenger RNA in the striatum of early grade Huntington's disease: a detailed cellular in situ hybridization study. *Neuroscience.* 1996; 72(4):1023–1036. [PubMed: 8735227]
34. Deng YP, Albin RL, Penney JB, Young AB, Anderson KD, Reiner A. Differential loss of striatal projection systems in Huntington's disease: a quantitative immunohistochemical study. *J Chem Neuroanat.* 2004; 27(3):143–164. [PubMed: 15183201]
35. Reiner A, Albin RL, Anderson KD, D'Amato CJ, Penney JB, Young AB. Differential loss of striatal projection neurons in Huntington disease. *Proc Natl Acad Sci U S A.* 1988; 85(15):5733–5737. [PubMed: 2456581]
36. Crossman AR, Mitchell IJ, Sambrook MA, Jackson A. Chorea and myoclonus in the monkey induced by gamma-aminobutyric acid antagonism in the lentiform complex. The site of drug action and a hypothesis for the neural mechanisms of chorea. *Brain.* 1988; 111 (Pt 5):1211–1233. [PubMed: 3179691]
37. Park SY, Kim HJ, Cho YJ, Cho JY, Hong KS. Recurrent hemichorea following a single infarction in the contralateral subthalamic nucleus. *Mov Disord.* 2009; 24(4):617–618. [PubMed: 19133654]
38. Guridi J, Obeso JA. The subthalamic nucleus, hemiballismus and Parkinson's disease: reappraisal of a neurosurgical dogma. *Brain.* 2001; 124(Pt 1):5–19. [PubMed: 11133783]
39. Lozano AM. The subthalamic nucleus: myth and opportunities. *Mov Disord.* 2001; 16(2):183–184. [PubMed: 11295767]
40. Lange H, Thorner G, Hopf A, Schroder KF. Morphometric studies of the neuropathological changes in choreatic diseases. *J Neurol Sci.* 1976; 28(4):401–425. [PubMed: 133209]

Figure 1. Anatomy of basal ganglia and the area of putamen assessed by stereology, and DARPP32 neuronal loss and atrophy in the putamen of Huntington's disease (HD) patients (A) A representative autopsy coronal brain tissue block at the level of the anterior commissure including the following structures: Head of the caudate nucleus (1), putamen (2), globus pallidus (3), internal capsule (4), corpus callosum (7), and anterior commissure (8). (B) A representative brain tissue block at the level of the mammillary body includes the following structures: caudate nucleus (1), putamen (2), globus pallidus external segment (3.1), globus pallidus internal segment (3.2), internal capsule (4), thalamus (5), mammillary body (6), corpus callosum (7). (C) Representative images from DARPP32 immunohistochemistry in the putamen of controls and HD patients (Mag: 40x). (D) Representative images from DARPP32 immunohistochemistry in the putamen of controls and HD patients (Mag: 100 x), bars indicate 100 μ m. There was progressive loss of neurites

and neuronal atrophy in HD brains. In the control (Con 1&2), dendrites appear studded with spines, and the neuropil also shows abundant punctate immunoreactivity. Cases of HD from Vonsattel I (VS-I) through Vonsattel IV (VS-IV) show increasing attenuation and loss of neurites, atrophy of the cell body but preservation of the nucleus and nucleolus. Note also the loss of neuropil punctate immunoreactivity. (E) Histogram of DARPP32 neurons counted in brains from 14 HD patients (range from VS II to VS IV) and 6 controls. A striking and significant loss of neurons is noted at all Vonsattel stages of disease. Values are means of neuronal number $\pm$ standard deviation (SD). ** $p < 0.01$ compared to the values in controls by one-way *ANOVA* with *Holm-Sidak post hoc* tests. (F) Overall putamen volume measured in 14 HD cases and 6 controls. The mean overall volume of putamen is reduced in all Vonsattel stages. Values are means of putamen volumes $\pm$ SD. ** $p < 0.01$ compared to the values in controls by one-way *ANOVA* with *Holm-Sidak post hoc* tests. (G) Cell volumes were measured in 14 HD cases and 6 controls. The mean neuronal volume is also reduced in all Vonsattel stages. Values are means of neuronal volumes $\pm$ SD. ** $p < 0.01$ compared to the values in controls by one-way *ANOVA* with *Holm-Sidak post hoc* tests. (H) Nuclear volumes were measured in 14 HD cases and 6 controls and showed no difference between the two groups. Values are means of nuclear volumes $\pm$ SD.

Figure 2. Neuronal loss in subthalamic nucleus in HD cases correlates with DARPP 32 neuronal loss in putamen

(A) Total STN neuronal numbers were examined on H&E-Luxol/Fast Blue stained sections of 7 controls and 10 HD cases. There is a significant neuronal loss in STN ($p < 0.05$) in the HD group by *Student's t*-test. Values are means of neuronal number $\pm$ SD. (B) STN overall volumes in 7 controls and 10 HD cases. There is a trend for atrophy in the HD group. Values are means of putamen volumes $\pm$ SD.

Figure 3. Correlations between DARPP32 neuronal loss in the putamen of HD patients and clinical motor abnormalities

Pearson Product Moment analysis shows (A) significant correlation between motor impairment score and DARPP32 neuronal numbers. (B) no correlation between chorea and DARPP32 neuronal numbers. (C) significant correlation between motor impairment score and volume of the putamen. (D) a trend for correlation between overall volume of putamen and DARPP32 neuronal numbers. (E) an inverse and significant correlation between motor impairment score and total number of neurons in subthalamic nucleus. (F) no correlation between chorea score and number of neurons in subthalamic nucleus.

Table 1

shows Demographic information of the HD and control groups

	Putamen		Subthalamic nucleus	
	Control	HD	Control	HD
N	6	14	7	10
Age (mean/year)	48.2±24.9	60.3±16.1	44.7±19.9	54.5±14.3
Gender (M vs F)	4 vs 2	6vs 8	3vs 4	5vs 5
Age range (years)	17–81	35–85	21–65	35–72

Table 2

provides individual clinical characteristics of 14 HD cases that were assessed for stereology study.

Case Number#	Gender	Age	CAG repeat	VS Grade	Post mortem delay (hours)	MIS	Chorea	Age of Onset	Interval Between Last Neurological Exam and Death (Months)	Cases with Entire STN Available *
Case 1	F	53	49	3	6	24	8	32	60	Yes
Case 2	F	48	50	3	31.5	8	13	27	119	
Case 3	M	47	43	2	20	2	10	39	102	Yes
Case 4	F	35	62	4	51	16	17	23	61	Yes
Case 5	M	74	41	2	4	6	9	69	47	Yes
Case 6	F	72	42	3	10	22	10	58	5	
Case 7	F	86	42	3	4	15	7	49	52	
Case 8	M	45	45	3	7	13	24	27	6	Yes
Case 9	M	72	42	3	8	17	13	49	30	Yes
Case 10	M	44	48	3	20	17	10	23	38	Yes
Case 11	F	86	41	3	18	35	13	53	21	
Case 12	F	65	44	4	14	25	14	45	79	Yes
Case 13	F	55	43	2	6	15	21	40	54	Yes
Case 14	M	63	46	3	6	7	8	42	147	Yes

Number of DARPP 32 neuron were estimated by stereology in all of these HD cases.

* Total neuronal numbers in subthalamic nucleus were estimated by stereology in these cases.

STN: subthalamic nucleus