The fate of striatal dopaminergic neurons in Parkinson’s disease and Huntington’s chorea

Philippe Huot,1, 2 Martin Lévesque1 and André Parent1

1Centre de Recherche Université, Laval Robert-Giffard and 2Département des Sciences Neurologiques, CHUQ-Hôpital de l’Enfant-Jésus, Québec, QC, Canada G1J 1Z4

Corresponding author: André Parent, Centre de Recherche Université Laval Robert-Giffard, 2601, de la Canadière, Local F-6500, Québec, QC, Canada G1J 2G3
E-mail: andre.parent@anm.ulaval.ca

The striatum harbours a population of dopaminergic neurons that is thought to act as a local source of dopamine (DA). This neuronal population increases in size in animal models of Parkinson’s disease, where striatal DA levels are low, but its fate in idiopathic Parkinson’s disease and Huntington’s chorea is poorly known. In this study, we used antibodies raised against the enzyme tyrosine hydroxylase (TH), a faithful marker of dopaminergic neurons, to compare, by means of stereological counting methods, the number of striatal TH+ neurons on post-mortem brain sections from Parkinson’s disease patients, Huntington’s disease patients and age-matched controls. Propidium iodide nuclear staining was also performed to avoid counting short TH+ axonal segments that bear a large swollen varicosity and resemble small bipolar neurons. In normal subjects, TH+ neurons were scattered throughout the striatum, but they abounded preferentially in the ventral portion of the structure and were more numerous in the putamen than in the caudate nucleus. They displayed a multipolar cell body of medium size (10–20 μm in diameter) that emitted 3–5 smooth dendrites, a typical characteristic of striatal interneurons. These TH+ cells were rarely found in the small TH-poor striosomes, most of them being embedded in the large TH-rich extrastriosomal matrix. The number of striatal TH+ neurons was also found to vary according to an inverse relation with the age of the subjects. In pathological brains, the morphological characteristics of the striatal TH+ neurons were relatively unaltered, but the number of such neurons was markedly reduced compared with controls. The striatum of Parkinson’s disease patients was found to contain six times less TH+ neurons than that of controls, whereas the striatum of Huntington’s disease patients was largely devoid of such neurons. These findings are at odds with the results obtained in rodent and monkey models of Parkinson’s disease, in which the number of striatal TH+ neurons is reported to increase markedly following DA denervation. Since Parkinson’s disease patients examined in this study were all treated with L-3,4-dihydroxyphenylalanine to compensate for the loss of striatal DA and that levels of striatal DA are reportedly higher in the striatum of Huntington’s disease patients compared with controls, we hypothesize that local DA concentrations exert a negative feedback on the expression of TH phenotype by striatal interneurons. A better knowledge of factors governing the in vivo state of this ectopic neuronal population could open new therapeutic avenues for the treatment of Parkinson’s disease and Huntington’s chorea.

Keywords: basal ganglia; striatum; neurodegenerative diseases; dopamine; striatal neurons

Abbreviations: DA = dopamine; DAT = DA transporter; L-dopa = L-3,4-dihydroxyphenylalanine; SN = substantia nigra

massive atrophy of the striatum due to the degeneration of striatal projection neurons, striatal interneurons being relatively spared in the disease (Dawbarn et al., 1985; Vonsattel et al., 1985; Cicchetti et al., 1996, 2000; Cicchetti and Parent, 1996; Petersen et al., 1999).

Evidence for the presence of dopaminergic neurons within the striatum was obtained in various species, including non-human and human primates, following immunohistochemical studies undertaken with antibodies directed against tyrosine hydroxylase (TH), the rate-limiting enzyme in DA synthesis, or against the DA transporter (DAT), another faithful marker of dopaminergic neurons (Dubach et al., 1987; Tashiro et al., 1989; Mura et al., 1995; Ikemoto et al., 1996, 1997; Betarbet et al., 1997; Betarbet and Greanamyre, 1999; Cossette et al., 1999, 2003, 2004, 2005a, b; Meredith et al., 1999; Porritt et al., 2000; Presna et al., 2000; Smith and Kieval, 2000; Mao et al., 2001; Nakahara et al., 2001; Palfi et al., 2002; Lopez-Real et al., 2003; Jollivet et al., 2004; Mazloom and Smith, 2006; Tandé et al., 2006). Detailed morphological and colocalization studies performed in human and non-human primates have revealed that the vast majority of striatal TH+ neurons express the enzyme glutamic acid decarboxylase (GAD) and exhibit smooth dendrites (Betarbet et al., 1997; Presna et al., 2000; Cossette et al., 2004, 2005a, b; Tandé et al., 2006); these neurons thus appear to represent a particular subtype of GABAergic striatal interneurons capable of producing both GABA and DA.

Interestingly, the number of striatal TH+ neurons was reported to increase markedly in parkinsonian rodents and monkeys compared with controls (Tashiro et al., 1989; Betarbet et al., 1997; Meredith et al., 1999; Mao et al., 2001; Palfi et al., 2002; Mazloom and Smith, 2006; Tandé et al., 2006), a finding that led to the suggestion that they could act as a local source of DA to compensate for the loss of this neurotransmitter that characterizes Parkinson’s disease (Betarbet et al., 1997; Bezard and Gross, 1998; Porritt et al., 2000; Cossette et al., 2003).

This conclusion was further supported by the results of an immunohistochemical study that reported a nearly 10-fold increase in the number of DAT+ neurons in the striatum of patients that had idiopathic Parkinson’s disease compared with controls (Porritt et al., 2000). In the latter study, however, most profiles that were counted are described as small (8–15 μm), unipolar or bipolar neurons, and about half of them were located outside the striatum, that is, within the pallidum and also within the internal capsule and ansa lenticularis. The morphological features of these DAT+ profiles and the extrastratal localization of about half of them indicate that a significant proportion of what was considered as small unipolar or bipolar neurons might instead have been short segments of swollen varicose dopaminergic fibres undergoing degeneration. Indeed, previous immunohistochemical studies revealed that the dopaminergic axons that innervate human basal ganglia are markedly varicose and reach the striatum by coursing through the internal capsule, the globus pallidus, as well as along the ansa lenticularis and the lenticular fasciculus (Cossette et al., 1999; Presna et al., 2000). Hence, because no nuclear markers were used, the number of DAT+ neurons might have been largely overestimated in the study with human Parkinson’s disease cases (Porritt et al., 2000).

In contrast to the situation in Parkinson’s disease, striatal DA levels were found to be significantly higher in Huntington’s disease compared with controls (Spokes, 1979, 1980; Bird, 1980; Bird et al., 1980). As previously mentioned, the interneurons are relatively spared in Huntington’s disease (Dawbarn et al., 1985; Vonsattel et al., 1985; Cicchetti et al., 1996, 2000; Cicchetti and Parent, 1996; Petersen et al., 1999). Since the vast majority of TH+ neurons in the striatum of both human and non-human primates appear to be interneurons (Betarbet et al., 1997; Presna et al., 2000; Cossette et al., 2005a; Mazloom and Smith, 2006; Tandé et al., 2006), it would be important to determine the fate of these elements in Huntington’s disease. Likewise, although the increase in striatal TH+ neurons noted in animal models of Parkinson’s disease has been confirmed by many investigators, the results obtained in the single investigation involving Parkinson’s disease patients need to be reassessed. We therefore thought it worthwhile to address this important issue by examining in detail the status of the striatal TH+ neuronal population with the help of post-mortem tissue from Parkinson’s disease and Huntington’s disease patients and age-matched controls.

Material and methods
Tissue collection
The post-mortem material used in this study came from the brains of 17 individuals: 4 patients who had Parkinson’s disease and were receiving L-3,4-dihydroxyphenylalanine (L-dopa) therapy together with 5 age-matched controls, and 4 patients who suffered from Huntington’s disease (grade 3–4/4) together with 4 age-matched controls (Table 1). The Parkinson’s disease and Huntington’s disease patients satisfied accepted clinical and neuropathological criteria for idiopathic Parkinson’s disease and Huntington’s chorea. Indeed, Lewy bodies and neuronal loss were documented within the SN of all of the Parkinson’s disease brains; the striatum of all of the Huntington’s disease patients satisfied accepted clinical and neuropathological criteria for idiopathic Parkinson’s disease and Huntington’s chorea. The 17 brains were sliced in half along the midline; one half of the brain was used for neuropathological examination, while the other half served for the present immunohistochemical investigation. The hemi-brains to be used for the present immunohistochemical investigation were sliced unfixed into 0.5 cm-thick slabs along the frontal or sagittal
HD4 were treated for immunohistochemical detection of TH. The entire anteroposterior extent of the striatum of brains PD 1 and PD 2 was dissected and immersed in a 10 mM citrate buffer solution (pH 6.0) for 2 days at 4°C. They were then immersed for 2 days in a 0.1 M phosphate-buffered saline (PBS, pH 7.4) solution containing 15% sucrose and 0.1% sodium azide. The slabs were then rinsed three times in PBS and placed in a 4% paraformaldehyde solution for 2 days at 4°C. After their removal from the storage medium, sections were rinsed three times in PBS and placed in a 0.1 M phosphate-buffered saline (PBS, pH 7.4) solution containing 2% NHS, 0.1% Triton, and the primary antibody directed against TH (1 : 500, mouse monoclonal antibody; ImmunoStar, Hudson, WI, USA). After three other rinses in PBS, the sections were incubated at room temperature for 1 h in a PBS solution containing 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by a similar citrate solution pre-heated to 75°C. Sections were then preincubated for 1 h in a PBS solution containing 5% normal horse serum (NHS), and 0.1% Triton X-100. This solution was replaced the next morning by an additional section plus one control section of brains C4, C8, HD1 and PD1. The only methodological differences with the protocol used for TH immunohistochemistry,
besides that of using an anti-DAT antibody (1:1000, rat monoclonal antibody; Chemicon) instead of an anti-TH antibody as the primary antibody, was the use of normal goat serum as the blocking serum and of goat-made anti-rat IgG as the secondary antibody.

Although PI is known to be a highly reliable marker of the nucleus in dead cells (Ockleford et al., 1981), its cellular penetration was nevertheless assessed on SN sections from brain HD₄, on which TH immunohistochemistry was also performed.

Material analysis
This study was entirely performed by means of immunofluorescent procedures and standard stereological methods. All immunostained sections were entirely scanned and fluorescence signals were imaged with a confocal laser-scanning microscope LSM 5 PASCAL (Zeiss, Oberkochen, Germany). The emission signals of Alexa 488 (TH/DAT) and PI were assigned the green and red colours, respectively. To be classified as a neuron, immunofluorescent profiles had to colocalize perfectly with the nuclear marker PI. Z-stack analyses were performed at 0.55–0.65 μm intervals and reconstructed orthogonal images were used to confirm the double-labelling of a structure. To be included in the count, immunofluorescent profiles had to be stained with the two markers (TH and PI) and to display at least two dendrites. Each TH+/PI+ profile was subjected to Z-stack analyses and orthogonal reconstructions to minimize the possibility of missing some neurons whose neurites were not entirely in the plane of section. Sections incubated without the primary antibodies remained virtually free of immunofluorescence and served as controls. Photomicrographs were handled with the Adobe Photoshop 7.0.1 software (Adobe, San Jose, CA, USA).

Statistical analysis
The number of TH+ neurons per section was calculated for each brain analysed in this study. Then, for each category of brains, the mean number of TH+ neurons per section, the standard deviation, the standard error of the mean, the variation coefficient, the median number of TH+ neurons per section, and the semi-interquartile interval were calculated (Dayhaw, 1969). To assess the relationship between age and the number of neurons, the coefficient of correlation (rho of Spearman, ρ) and the determination coefficient were calculated. Statistical differences between the groups were assessed with the Mann–Whitney U test. Differences were considered statistically significant if P < 0.05 (Siegel, 1956; Dayhaw, 1969). The statistics were computed using the SPSS 13.0 for Windows (SPSS, Chicago, IL) and Microsoft Office Excel 2003 (Microsoft Canada, Mississauga, ON, Canada) softwares.

Results

Technical considerations
The reliability of PI as a nuclear marker was assessed on sections through the SN, because this structure, particularly its pars compacta, contains a very large number of closely packed TH+ neurons (Parent, 1996), which makes it an ideal substrate to validate PI as a nuclear marker of TH+ neurons in post-mortem human brain. A detailed examination of SN sections revealed that >97% of nigral neurons that expressed TH had their nucleus stained with PI (Fig. 1). This finding shows that PI has a high degree of penetration in TH+ neurons and that it can be used as a faithful nuclear marker in post-mortem human brain tissue. These sections contained numerous PI-labelled nuclei that were not surrounded by a TH+ cytoplasm. These labelled nuclei probably belong principally to pericellular glial cells and, to a lesser extent, to some non-dopaminergic neurons (GABAergic neurons) scattered among the TH+ neurons of the pars compacta of the SN.

Striatal TH+ neurons in normal brains
The vast majority of TH+ neurons encountered in the striatum of normal individuals had a perikaryon of small to medium size (diameter ranging from 10 to 20 μm) that emitted at least three smooth dendrites; only a few TH+ neurons displayed a bipolar shape or had a diameter >20 μm (Fig. 2). These neurons were present throughout the anterior striatum, but they were slightly more abundant in the ventral striatum and occurred in larger number in the putamen than in the caudate nucleus. Such neurons were absent from extrastriatal sites, such as the internal capsule. These data are in accordance with the findings of our earlier study in which the morphological characteristics and distributional patterns of human striatal TH+ neurons were studied in a different set of individuals (Cossette et al., 2005a).

In all mammals, including humans, the striatum can be divided into two main compartments termed striosomes (or patches) and extrastriosomal matrix, each having a specific chemical composition and a distinct set of anatomical projections (Graybiel, 1990; Prens et al., 1999; Cicchetti et al., 2000). The extrastriosomal matrix occupies about 85% of the total striatal volume, whereas the much smaller and patchier striosomes form a complex three-dimensional...
network embedded within the extrastriosomal matrix (Johnston et al., 1990). In the anterior striatum, the neuropil at the level of the extrastriosomal matrix is markedly enriched in TH, whereas the activity of this enzyme is weak in striosomes (Ferrante and Kowall, 1987; Graybiel, 1990; Prensa et al., 1999, 2000; Cicchetti et al., 2000). This chemical difference between striosomes and extrastriosomal matrix was used in this study to determine the location of striatal TH+ neurons with regard to the two main striatal compartments. We found that the vast majority of TH+ intrastriatal neurons (n = 29/30 cells for which this was verified) were located within the matrix compartment.

Also of interest was the finding that the median number of TH+ neurons in Huntington’s disease control brains was higher than that in Parkinson’s disease control brains (Fig. 3). Since the individuals that formed the Huntington’s disease control group were younger than those of the Parkinson’s disease control group, this finding suggests that the number of striatal TH+ neurons might decrease when the age of the subjects increases. This hypothesis was supported by the calculation of the r value and that of the coefficient of determination, which were 0.63 (P = 0.035) and 0.39, respectively (Fig. 4).

**Fig. 4** Scatter plot showing the relationship between age and the number of striatal TH+ neurons per section, in normal brains. The regression line indicates an inverse relation between the number of striatal TH+ neurons per section and age. $r = -0.63$ ($P = 0.035$).

**Fig. 3** (A) Table summarizing the number of TH+ neurons per striatal section in normal, Parkinson’s disease, and Huntington’s disease brains. The number of such neurons is significantly smaller in both Huntington’s disease and Parkinson’s disease brains compared with age-matched controls. The difference is more pronounced in Huntington’s disease brains, where very few striatal DA neurons were present. (B) Bar diagram comparing the median number of striatal TH+ neurons in the different types of brains; note that the number of such neurons is much smaller in the two categories of pathological brains compared with their respective controls. In the study, the median number and the Q are preferred over the mean and the SD or SEM as central tendency and dispersion measure, respectively, because we obtained a V value that was >0.15, which means that the results were too dispersed to accurately use the latter three modalities (Dayhaw, 1969). Error bars: Q.

**Striatal TH+ axonal varicosities**

Sections from both normal and pathological brains displayed a multitude of immunofluorescent profiles resembling unipolar or bipolar neurons (Fig. 2). However, none of these TH+ profiles colocalized with the nuclear marker PI. These immunoreactive profiles without a nuclear structure probably correspond to short axonal segments endowed with swollen varicosities, some of which can easily reach 10–15 μm in diameter, that is, the size of the perikaryon of small TH+ neurons. These TH+ varicosities were far more numerous than TH+ neurons with a PI+ nucleus and they occurred in all sectors of the striatum. Although present in all the brains examined, they predominated in the striatum of Parkinsonian patients and, to a lesser extent, in the elderly individuals. Their predominance in Parkinsonian brains might reflect the presence of numerous nigrostriatal TH+ axons that display markedly swollen varicosities while undergoing degeneration in such a disease. These findings point to the necessity of using a nuclear marker to avoid confusion between varicose axonal profiles and true bipolar neurons and to ensure that non-neuronal profiles are not included in the count.
Striatal TH+ neurons in pathological brains

A severe TH+ denervation affecting principally the dorsal striatum (caudate nucleus and putamen) and much less so the ventral striatum (nucleus accumbens and surrounding structures) was noted in the four Parkinsonian brains examined. By comparison to age-matched controls, the density of TH+ axonal profiles distributed throughout the caudate nucleus and putamen were greatly reduced in Parkinson’s disease cases, most likely as a result of the degeneration of nigrostriatal dopaminergic neurons. Similarly, the number of TH+ neurons intrinsic to the striatum was significantly reduced in Parkinson’s disease cases; the median number of TH+ neurons per section in controls was 0.64 ± 0.06, compared with 0.10 ± 0.03 in Parkinsonian patients, which corresponds to a >6-fold decrease ($P = 0.032$) (Fig. 3A).

In contrast, TH+ fibres were abundant throughout the striatum and the neuropil was intensely labelled in Huntington’s disease cases. Yet, despite this discrepancy between Huntington’s disease and Parkinson’s disease brains with respect to the striatal TH+ innervation, very few TH+ neurons were disclosed in the striatum of Huntington’s disease brains, as in Parkinson’s disease cases. In fact, only four TH+ neurons were detected in the four Huntington’s disease brains, so that the median number of striatal TH+ neurons per section in Huntington’s disease brains was 0.00 ± 0.02, compared with 1.04 ± 0.11 in age-matched controls ($P = 0.029$). Hence, the decrease in the number of striatal TH+ neurons in Huntington’s disease patients was even more impressive than in Parkinson’s disease cases, but the difference between the values obtained in the two pathological groups (Huntington’s disease and Parkinson’s disease) was not statistically significant ($P = 0.343$) (Fig. 3A).

The neuronal reduction witnessed in the pathological groups was also perceptible on an individual basis. Indeed, the pathological brains, when taken individually, harboured less TH+ neurons per section than the age-matched controls (data not shown). As in age-matched controls, TH+ neurons were encountered in all sectors of the striatum in Parkinson’s disease and Huntington’s disease patients, but their number was much too small to determine if they predominated in any given specific area.

In sections immunostained with anti-DAT antibodies, the results were in accordance with those obtained in sections treated with anti-TH antibodies. For example, when compared with age-matched controls, the neuropil’s immunofluorescence was reduced in striatum of Parkinson’s disease patients, whereas it was slightly increased in that of Huntington’s disease patients. In addition, no DAT+ neurons were encountered in the striatum of both Parkinson’s disease and Huntington’s disease patients, while such neurons were present in the striatum of control brains (data not shown), a finding that is congruent with the results obtained by TH immunohistochemistry. No quantitative estimates of DAT+ neurons were made since the number of sections immunostained for DAT was too small and this experiment served essentially as a means to validate the data obtained with TH as a neuronal marker of striatal DA neurons.

Discussion

This study demonstrates that, in both Parkinson’s disease and Huntington’s chorea, the number of TH+ intrastratial neurons is significantly smaller than that in age-matched controls. In addition, our results demonstrate that TH+ neurons are preferentially located within the extrastriosomal matrix compartment of the striatum and that their number decreases as age increases. The functional significance of these results will now be discussed in the light of data obtained in various species and different conditions.

Striatal TH+ neurons in pathological brains

The presence of striatal TH+ interneurons has been reported in rodents, monkeys and humans and their number was shown to increase in DA-depleted conditions, such as those encountered in rodent and non-human primate models of Parkinson’s disease (see references in the Introduction section). The latter finding led to the concept that these neurons act as a local source of DA to compensate for the loss of this neurotransmitter in DA-denervated conditions (Betarbet et al., 1997; Bezard and Gross, 1998; Porritt et al., 2000; Cossette et al., 2003). The fact that TH+ neurons are virtually absent from the striatum of Huntington’s disease patients, as demonstrated in this study, is congruent with such a view. Indeed, since striatal DA levels are significantly increased in Huntington’s disease cases compared with controls, sometimes by as much as 87% (Spokes, 1979, 1980; Bird, 1980; Bird et al., 1980), supplemental production of DA by striatal interneurons in such a DA-rich environment appears unnecessary and could even exacerbate the symptomatology of the disease.

Two of the Huntington’s disease brains used in this study were intensely stained for TH, whereas the two others were less intensely labelled. However, very few TH+ neurons were detected in the striatum of Huntington’s disease brains, including those that were markedly stained for the enzyme. We therefore used an antigen retrieval procedure on the two brains that stained less well from the start. This finding reveals that the numerical decrease of TH+ neurons in Huntington’s disease brains is a genuine phenomenon and not the consequence of a technical problem in the detection of these immunoreactive neurons.

In contrast, the fact that striatal TH+ neurons were much less numerous in Parkinsonian patients than in age-matched
controls is at odds with the notion that these neurons are part of a compensatory DA mechanism. Since striatal DA levels are significantly decreased in Parkinson’s disease (Ehringer and Hornykiewicz, 1960; Hornykiewicz and Kish, 1986), as confirmed by the severe loss of striatal TH+ fibres and axon terminals noted in all Parkinsonian striata examined in this study, the reduction in the number of TH+ neurons is unexpected and contradicts the results obtained previously in parkinsonian rats, monkeys and humans (Tashiro et al., 1989; Betarbet et al., 1997; Meredith et al., 1999; Porritt et al., 2000; Palfi et al., 2002; Mazloom and Smith, 2006; Tandé et al., 2006). Such a discrepancy is unlikely to be due to a methodological bias, because most studies were undertaken with a similar immunohistochemical method, which was applied to both normal and pathological individuals. However, the fact that we used stringent inclusion criteria, such as the presence of a PI+ nucleus and at least two clearly visible dendrites in our analysis of the immunoreactive profiles, might have led to an underestimation of the striatal TH+ neurons. However, because the same rigorous criteria were applied to both normal and pathological brains, this has not affected our comparison—and, therefore, the ratio of neurons—between normal, Parkinson’s disease and Huntington’s disease brains. The use of the nuclear marker PI+, however, virtually precluded the inclusion of axonal varicosities of degenerating nigrostriatal neurons in our sample; their exclusion probably accounts for the discrepancy between our results and those obtained in Parkinson’s disease patients by Porritt et al. (2000).

All Parkinsonian brains used in this study were receiving l-dopa, the immediate precursor of DA that is metabolized into DA in brain tissue (Lloyd et al., 1975; Standaert and Young, 2006), which was not the case in studies with animal models of Parkinson’s disease. Hence, the low number of striatal TH+ neurons encountered in Parkinsonian brains compared with age-matched normal individuals, which is at odds with the increase in the number of such neurons in Parkinson’s disease animal models compared with controls (Tashiro et al., 1989; Betarbet et al., 1997; Meredith et al., 1999; Palfi et al., 2002; Mazloom and Smith, 2006; Tandé et al., 2006), could be due to the fact that striatal DA depletion in Parkinsonian patients was exogenously compensated by l-dopa. Therefore, the amount of striatal DA, whether endogenously supplied by nigrostriatal neurons, or exogenously provided via l-dopa, might play a key role in regulating the number of striatal TH+ neurons which could, indeed, act as a local DA source, in an uncompensated DA-deprived environment. It may thus be hypothesized that the number of striatal TH+ neurons is inversely related to the DA levels in the striatum.

The effect of l-dopa on the numerical regulation of TH+ neurons nevertheless remains controversial. Indeed, two studies performed in rats rendered parkinsonian following 6-hydroxydopamine (6-OHDA) administration reported that l-dopa could increase the number of striatal TH+ cells compared with healthy animals and/or to l-dopa-untreated parkinsonian rats. In these studies, Parkinsonian animals received either a single 100 mg/kg intraperitoneal (IP) dose of l-dopa or a chronic IP daily dose of 50 mg/kg of l-dopa (Lopez-Real et al., 2003; Jollivet et al., 2004). This greatly exceeds the usual oral daily doses taken by human Parkinson’s disease subjects (Lang and Lozano, 1998; Shannon, 2004) and could account for the discrepancy between our results and those of these two studies.

We thus hypothesize that l-dopa (or DA itself) could have trophic effects, as demonstrated in an in vitro study in which l-dopa and DA were found to induce a TH+ phenotype (Du and Iacovitti, 1995). However, we believe that this effect depends on the concentration of the transmitter. There could be a threshold above which l-dopa/DA may exert a trophic effect, by a mechanism still unknown; under this threshold, l-dopa/DA would not exert this trophic effect and would regulate TH expression/activity via a negative feedback mechanism (Kumer and Vrana, 1996). The idea of a threshold necessary for some growth factors to exert their trophic effect on the regulation of TH expression was suggested by a few in vitro studies (Du and Iacovitti, 1995, 1997a,b; Du et al., 1995). It remains unclear, however, if this potential trophic effect applies to both primates and rodents. Indeed, the in vitro experiments mentioned above were performed on murine striatal cell culture and there could be some phenotypic differences between striatal TH+ neurons in primates and rodents. For example, the enzymes TH and GAD67 are coexpressed in human and non-human primates (Betarbet et al., 1997; Cossette et al., 2005b; Tandé et al., 2006), whereas Jollivet et al. (2004) were unable to demonstrate such a colocalization in rats. Hence, there could be some fundamental neurochemical differences between TH+ neurons in rodents and primates, which could thus react differently to the same stimulus. Clearly, other studies are needed to clarify the potential TH-inducible properties of high-dose l-dopa treatment.

The fact that striatal TH+ neurons are more numerous in DA-denervated conditions than in a normal state could be due to the production of new neurons that progressively develop the TH+ phenotype or to a phenotypic change of pre-existent striatal mature neurons. The second explanation appears more likely since, although neurogenesis occurs throughout life in the striatum of adult primates (Bédard et al., 2002), studies in mice (Mao et al., 2001) and aged macaques (Tandé et al., 2006) reported that lesions of the nigrostriatal pathway did not induce striatal neurogenesis. The results of these investigations suggest that the high number of TH+ neurons observed in a DA-depleted striatum rather results from pre-existent neurons adopting a TH+ phenotype. Such phenotypic changes were reported in the hypothalamic arcuate nucleus of rats, following lesions of the DA tuberoinfundibular system (Ershov et al., 2005). In mice, several striatal neurons that did not express the TH protein to a level detectable with immunohistochemistry were nevertheless found to express a significant...
level of TH mRNA with in situ hybridization procedures (Baker et al., 2003). Hence, it may be postulated that such neurons could develop a TH+ phenotype in DA-deprived conditions and that this phenotype could be repressed once DA levels have returned to normal.

In Huntington’s disease, where levels of striatal DA are reportedly higher than in controls, striatal TH+ neurons might have down-regulated their TH+ phenotype to a point where they became undetectable with the immunofluorescent approach used in this study. As mentioned above, pathological processes at play in Huntington’s disease target principally striatal projection neurons, but certain interneurons are also affected, to a lesser degree, in the disease (Cicchetti and Parent, 1996; Petersen et al., 1999; Cicchetti et al., 2000). However, we do not know if TH+ interneurons die or simply modulate their chemical phenotype as a response to the degenerative pressures that occur in the disease.

Striatal TH+ neurons in relation with age

The numerical reduction of TH+ neurons with increasing age noted in this study can hardly be explained by an increase in ambient DA, since striatal DA decreases with age, due to an age-related death of nigral dopaminergic neurons (Carlsson, 1987). However, it is important to mention that a p of —0.63 implies a link of moderate strength between two variables—here, subject’s age and the number of striatal TH+ neurons—and that such a link must be interpreted cautiously. In addition, a coefficient of determination of 0.39 means that age can explain up to 39% of the reduction of the number of TH+ neurons, the remaining 61% depending on other factors, as yet unidentified, but possibly age-related (Siegel, 1956; Dayhaw, 1969; Huot, 2003). Hence, the decrease in the number of striatal TH+ neurons might simply parallel the normal neuronal loss that happens in the ageing striatum (Esiri et al., 1997), but other factors are also probably involved. Perturbations in the concentrations of some neurotrophic factors like brain-derived neurotrophic factor or other molecules such as bone morphogenic proteins, protein kinase A and C activators or the muscle-derived differentiation factor, that appear to play a role in the induction of a TH+ phenotype in murine striatal neurons in culture (Iacovitti, 1991; Du et al., 1995; Du and Iacovitti, 1997a, b; Stull et al., 2001), could be involved in this age-related reduction. Variations in the levels of such neurotrophic factors with ageing (Hattiangady et al., 2005; Hwang et al., 2006) could possibly preclude the phenotypic transformation of striatal cells into TH+ neurons and hence force the striatum to use other compensatory mechanisms. One of these mechanisms could be an increase of the activity of the remaining nigrostriatal neurons. Indeed, midbrain nigral neurons possess an impressive capacity to increase their neurotransmitter production to compensate for the loss of their congener (Carlsson, 1987). Other possible compensatory mechanisms are the up-regulation of D2 receptors and/or enkephalinergic expression within the striatal projection neurons, or an increase in the electrophysiological activity of the subthalamic nucleus and the internal segment of the globus pallidus, which might also play a role in the preclinical stages of Parkinson’s disease (Bezard et al., 2003). Further studies are obviously needed to better understand the role of these non-DA compensatory mechanisms in relation with the increase in striatal TH+ neurons, which might constitute the ultimate compensatory mechanism.

Striatal TH+ neurons and the matrix compartment

The preferential location of TH+ neurons in the extrastriosomal matrix could simply reflect the fact that this striatal compartment is much more voluminous and thus harbours a much larger number of striatal neurons than the smaller striosomal compartment (Graybiel, 1990; Johnston et al., 1990; Prensa et al., 1999). However, the predominance of TH+ neurons in the matrix is congruent with the fact that nigrostriatal dopaminergic axons arborize more profusely in the extrastriosomal matrix than in striosomes (Graybiel, 1990; Parent et al., 1995; Prensa et al., 1999; Prensa and Parent, 2001). Both sets of data indicate that DA-related mechanisms affect preferentially, but not exclusively, matriceal neurons. This finding has important functional implications, since matriceal and striosomal neurons do not share the same combinations of afferent and efferent connections (Parent and Hazrati, 1995). In any events, the heterogeneity noted in this study in regard to the distribution of striatal TH+ neurons as well as that of TH+ fibres and axon terminals within the striatal neuropil should be kept in mind when interpreting the pattern of DA denervation that characterizes the various stages of Parkinson’s disease.

Concluding remarks

This study has confirmed the presence of TH+ neurons intrinsic to the striatum in human. It has also shown, for the first time, that the elements of this ectopic neuronal population are preferentially located in the extrastriosomal matrix and that their number decreases with advancing age. Furthermore, the number of these striatal TH+ neurons was found to be significantly smaller in Huntington’s disease patients and in L-dopa-treated Parkinsonian patients than in age-matched controls. This finding leads to the suggestion that the number of striatal neurons that develop the TH+ phenotype is regulated by the local concentrations of DA. Other regulatory factors are also probably involved. Indeed, in vitro experiments showed that several factors can induce a TH+ phenotype in neurons (Iacovitti, 1991; Du et al., 1995; Du and Iacovitti, 1997a, b; Stull et al., 2001), and lentivirally delivered glial cell line-derived neurotrophic factor was found to markedly increase the number of striatal TH+ neurons in the striatum of Parkinsonian monkeys (Palﬁ et al.,
Dopaminergic neurons in Parkinson’s and Huntington’s diseases


2002). Hence, the identification and characterization of factors able to modulate in vivo the number of striatal TH+ neurons could open new avenues for the treatment of Parkinson’s disease.

Acknowledgements

The authors express their gratitude to Dr Emmanuelle Porcher, for having provided some of the brains used in the study, to Dr Peter V. Gould, for the neuropathological analysis of the brains, and to Mrs. Doris Côté, for helpful technical support. This research was supported by the grant MT-5781 of the Canadian Institute for Health Research.

References


Du X, Stull ND, Iacovitti L. Brain-derived neurotrophic factor works coordinately with partner molecules to initiate tyrosine hydroxylase expression in striatal neurons. Brain Res 1995; 680: 229–33.


Ikemoto K, Menei P. Striatal tyrosine hydroxylase immunoreactive neurons are induced by l-dihydroxyphenylalanine and nerve growth factor treatment in 6-hydroxydopamine lesioned rats. Neurosci Lett 2004; 362: 79–82.