Long-Term Cortical Atrophy after Excitotoxic Striatal Lesion: Effects of Intrastriatal Fetal-Striatum Grafts and Implications for Huntington Disease

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Abstract. It is not currently clear whether the cortical atrophy observed in Huntington disease (HD) is entirely a direct consequence of the disease or at least partially a secondary consequence of striatal atrophy. This is of major importance for evaluating the possible therapeutic value of intrastriatal fetal-striatum grafts in HD. Cresyl violet-stained sections from rats that had received striatal excitotoxic lesions 1 wk or 4 wk previously showed small and statistically nonsignificant decreases in the thickness of cortical layers V and VI, while series from rats lesioned 12 months previously showed marked decreases in the thickness of the whole cortex (~35% decrease), layer V (~45%–50%) and layer VI (~45%–50%), together with marked neuron loss in these layers. In deep layer V and layer VI, Fluoro-Jade staining showed labeled neurons in animals lesioned 1 wk previously, labeled neurons and astrocytes in animals lesioned 4 wk previously, and practically no labeling in animals lesioned 12 months previously. Intracortical injection of Phascolus vulgaris leucoagglutinin revealed that corticostriatal fibers were practically absent from the lesioned area of striata lesioned 12 months previously. However, rats that received intrastriatal fetal-striatum grafts shortly after the lesion and were killed 12 months later showed a significant reduction in cortical atrophy, and a large number of labeled corticostriatal fibers surrounding and innervating the graft. In addition, a reduction in the number of Fluoro-Jade-labeled cells in the cortex was already apparent at 3 wk post-grafting. Regardless of whether HD has a primary effect on the cortex, the present results suggest that the striatal degeneration caused by HD contributes markedly to the cortical atrophy, and that intrastriatal grafts may ameliorate this secondary component of the cortical degeneration.

Key Words: Cortex; Cortical atrophy; Grafts; Huntington disease; Neurodegeneration; Striatum; Transplantation.

INTRODUCTION

Huntington disease (HD) is an autosomal dominant familial neurodegenerative disorder of mid-life onset, characterized by progressive motor impairment with psychiatric and cognitive deterioration. The neuropathologic hallmarks of HD are atrophy and progressive shrinkage of the striatum. The medium spiny projection neurons are the most directly affected, with the other neuronal populations being relatively spared, and the neuronal degeneration is accompanied by strong astroglial reactivity (1). However, other regions of the brain, including the globus pallidus, the subthalamic nucleus, the substantia nigra (pars reticulata), some regions of the thalamus, and particularly the cerebral cortex, also show atrophy (2–7). Since these regions are connected with the striatum, it is not clear whether their degeneration is only a direct consequence of the disease or at least partially a secondary consequence of the striatal atrophy. Clarification of this question is of great interest for understanding the mechanisms involved in the development of HD, and particularly for assessing whether replacement of the degenerated striatal neurons is likely to be of value for treating HD (10, 11). Given that the most marked extrastriatal atrophy has been observed in the cortex, and that cortical atrophy seems to be related to the symptoms that are the most significant cause of suffering for patients and families (i.e. “frontal system” dementia [12]), this question is of particular interest in relation to the cortex.

Gross atrophy of the cerebral cortex is typically observed in autopsied brains of patients with HD (3, 7). However, some authors have reported no neuron loss in the cortex in HD patients (11, 12). More recent studies taking into account the atrophy-induced cortical shrinkage (2, 5, 7) have shown a loss of large pyramidal neurons in cortical layers III, V and especially in layer VI in HD brain, and a corresponding decrease in the thickness of these layers. Since only a subset of pyramidal cells are lost, the possibility of retrograde degeneration of cortical neurons that project solely or principally to the striatum (i.e. cortical atrophy secondary to striatal atrophy) has been suggested (7). A primary cortical lesion has also been proposed; however, the mechanisms involved have not been clarified, since the level of huntingtin in cortical neurons, the density NMDA receptors in such neurons, and other candidate predictors have failed to explain why certain types of neurons die (13, 14). Probably, both primary and secondary mechanisms are involved. Primary mechanisms may result unaffected by striatal grafts. However, these grafts could counteract a secondary cortical atrophy. The possible existence of cortical retrograde degeneration after striatal atrophy and the effects of intrastriatal grafts on this cortical atrophy have been studied in the present experiments.

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Various animal models of HD have been developed, including intrastriatal injection of excitotoxins (15, 16), systemic injection of 3-nitropropionic acid (17), and transgenic mouse models (1, 18). In the later 2 models, however, there may be direct effects on cortical neurons (19, 20). Intrastriatal injection of ibotenic acid (IA) is a useful tool for studying the response of cortical neurons to extensive excitotoxin-induced striatal degeneration in the absence of other factors (i.e. primary mechanisms) that may directly induce loss of cortical neurons. A large number of previous studies in rats have shown that fetal striatal tissue grafted in excitotoxically lesioned striatum can partially counteract the lesion-induced behavioral (21) and biochemical (22) deficits, and can establish afferent and efferent connections with the host (23–26). Particularly, cortico-striatal fibers innervate the graft (23, 24, 27, 28) and induce functional responses in the grafted neurons (29–31). In studies of this type, the grafts are typically implanted in the acutely lesioned striatum (i.e. 1–4 wk after lesion), and their functional effects may be related not only to replacement of striatal connections but also to protection against secondary degeneration of extrastriatal regions, particularly the cerebral cortex, by providing postsynaptic target cells for afferent fibers.

If the cortical atrophy observed in HD is indeed mostly or at least significantly a consequence of previous striatal atrophy, and if intrastriatal grafts are able to protect cortical neurons against retrograde degeneration, then intrastriatal grafting can be expected to be highly beneficial. If not, the prospects of significant improvement after grafting are clearly reduced. In the work reported here, we studied retrograde cortical degeneration for 12 months after excitotoxic striatal lesion using Nissl staining, Fluoro-Jade (a marker for neuronal degeneration), and intra-cortical injections of PHA-L (Phaseolus vulgaris leucoagglutinin). In addition, we used the same methods to investigate the effects of intrastriatal fetal-striatum grafts on cortical degeneration.

MATERIALS AND METHODS

Experimental Design

A total of 58 female Sprague-Dawley rats (weighing ∼200–250 g at the beginning of the experiment) were used. They were divided into 6 groups (A–F). Group A (control group; n = 8) was untreated. Rats in group B (acute lesions) received unilateral (B1; n = 8) or bilateral (B2; n = 4) intrastriatal injections of ibotenic acid (IBO) (Sigma, St. Louis, MO) and were killed 1 wk later. Rats in groups C and D received unilateral injections of IBO and were killed 4 wk later (group C; subacute lesions; n = 8) or 12 months later (group D; chronic lesions; n = 12). Finally, rats in groups E and F received unilateral intrastriatal injections of IBO and, 1 wk later, a cell suspension from fetal striatal primordia in the same striatal area, and were killed 1 yr later (group E; long-term grafts; n = 8) or 3 wk later (group F; short-term grafts; n = 10). In each group, (except group B2) 3–4 rats received frontal cortical injections of the anterograde tracer Phaseolus vulgaris leucoagglutinin 10–14 days before being killed, to enable study of corticostriatal afferents. After the corresponding survival times, the rats were killed and processed for cresyl violet and Fluoro-Jade histochemistry, or PHA-L immunohistochemistry.

Excitotoxic Lesion and Transplantation

A total of 14 µg of ibotenic acid (10 µg/µl in 0.1 M phosphate buffer, pH 7.4) was injected into the right striatum, at 3 injection sites: (I) A = +0.2, L = 3, V = 5.5; (II) A = +0.2, L = 3, V = 4; (III) A = +1.5, L = 2.5, V = 4.7 (A is anterior from bregma, L = lateral from bregma, V = ventral from dura; tooth bar at −2.3; all coordinates in mm). One week post-lesion, the rats in groups E and F received intrastriatal injections of cell suspensions prepared from striatal primordia obtained at gestation day 14–15 (E 14–15), as follows. The lateral gangli- onic eminences were dissected out and incubated in DMEM (Gibco, Paisley, Scotland) containing 0.1% trypsin (Sigma) and 0.05% DNase (Sigma), for 20 min at 37°C. Afterwards, the tissue was rinsed in DNase/DMEM and mechanically dissociated to produce a milky cell suspension. This cell suspension was centrifuged at 600 rpm for 5 min, and the supernatant was carefully removed and resuspended in 0.05% DNase/DMEM to the final volume required. Approximately 1 million viable cells (estimated by acridine orange/ethidium bromide (Sigma) were administered to each rat at 3 injection sites: (I) A = +0.2, L = 3, V = 4.5; (II) A = +0.6, L = 2.7, V = 4.5; (III) A = +1.5, L = 2.5, V = 4.7 (tooth-bar at −2.3).

Cresyl Violet and Fluoro-Jade Staining

After the corresponding survival times, the animals were deeply anesthetized with chloral hydrate (Merck, Damstadt, Germany), 400 mg/kg, and preperfused with 0.9% saline for 30 s via the ascending aorta, followed by fixation with cold 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 (Merck). The brains were then removed, washed and cryoprotected in the same buffer containing 20% sucrose (Merck), and finally cut into 40-µm sections on a freezing microtome. Series of sections were then processed for cresyl violet (Merck) or Fluoro-Jade staining. Fluoro-Jade staining was performed essentially following the procedure of Schmued et al (32). Briefly, sections were mounted with distilled water onto gelatin-coated slides (Merck), dried and immersed in 100% ethanol (3 min) then 70% ethanol (1 min) and distilled water (1 min). The slides were then transferred to a 0.06% solution of potassium permanganate (Merck) for 15 min with gently shaking on a rotating platform, then rinsed for 1 min in distilled water, then transferred to a solution of Fluoro-Jade (Histo-Chem Inc., Jefferson, AR) (0.001% in 0.1% acetic acid) for 30 min. After staining, the sections were rinsed 3 times in distilled water, dehydrated, immersed in xylene, and then coverslipped with D.P.X. (BDH, Poole, England) mounting medium.

PHA-L Injections and Immunohistochemistry

Ten to 14 days before being killed, half of the rats in each group received multiple (15–20) ionophoretic injections of PHA-L (Vector, Burlingame, CA) (2.5% dissolved in 0.1 M phosphate-buffered saline, pH 7.4) into the frontal cortex ipsilateral to the lesion, 1–2 mm below the dura, as described in...
Fig. 1. Thickness of whole cortex, layer V, and layer VI in control rats (group A), in rats lesioned a week previously (AC-LE, acute lesion, group B), 1 month previously (SAC-LE, subacute lesion, group C) or 12 months previously (C-LE, chronic lesion, group D), and in rats with long-term grafts (C-GRRAFT, group E) or short-term grafts (AC-GRRAFT, group F). The data were normalized to those obtained in control animals and are represented as mean ± SEM. Means that do not differ significantly are indicated by the same letter (p < 0.05, one-way ANOVA and post-hoc Tukey test).

Quantification and Data Analysis
The thicknesses and number of neurons of the cerebral cortex layers were measured in cresyl violet-stained sections with the aid of NIH Image 1.55 image analysis software (Wayne Rasband, MIMH) on a Macintosh personal computer coupled to a videocamera (CCD-72, MTI, Michigan City, IN) connected to an Optiphot 2 microscope (Nikon, Tokyo, Japan). At least 3 nonconsecutive sections through the central striatum were taken per rat, and thickness and number of neurons were estimated for each cortical layer and the whole cortex at a level between the frontal area 1 and the parietal area 1 (i.e. at the level of the forelimb area) (34, 35). Areas close to the needle tracts were excluded. Neurons were distinguished from glial cells on morphological grounds, and in each cortical layer we counted all neurons with visible nuclei that were totally contained within a 0.0825 mm² (500 × 165 μm) area, as well as all neurons crossing the upper or left boundary of this area; neurons crossing the lower or right boundary were not counted. The counts on the side ipsilateral to the lesion were normalized to the counts on the control cortex. Whether counts varied among groups was investigated using ANOVA followed by post hoc Tukey tests (p < 0.05). Data normality and homogeneity of variances were confirmed before each ANOVA. All statistical analyses were performed using the package Sigmastat 2.0 (Jandel Scientific, San Diego, CA).

RESULTS

Group A (Control)
In sections stained with cresyl violet, the 6 cortical layers were identified (34), allowing estimation of the thickness and cell density of each layer. In Fluoro-Jade-stained sections, no consistent labeling was observed in the cortex or striatum. After PHA-L injections in the frontal cortex, a dense network of anterograde labeled fibers was observed bilaterally in the striatum as described in detail previously (23, 24, 36). The labeled fibers could be followed through the cortex, corpus callosum, external capsule and bundles of the internal capsule, and finally innervated the striatum. A dense network of thin varicose-labeled fibers was observed bilaterally in the striatum, though predominantly ipsilateral to the injection site.

Group B (Acute Lesions)
One week after unilateral excitotoxic lesion (group B1), no significant gross shrinkage of the striatum was observed. However, the lesioned striatum showed a dramatic loss of neurons and a marked increase of glial cells. With respect to control group values, small decreases were observed in cortex thickness, layer-V thickness, and layer-VI thickness; however none of these decreases was statistically significant (Fig. 1). Likewise, mean individual-layer cell densities in no case differed significantly from the corresponding control group mean. In Fluoro-Jade-stained series, intense staining was observed in the lesioned area of the striatum, which showed abundant intensely labeled glial cells and degenerated neuronal somata and processes (Fig. 2). In striatal areas adjacent to
the lesion (which appeared unaffected in cresyl violet-stained sections), numerous intensely stained neuronal somata were observed. In addition, Fluoro-Jade-positive neurons were observed in extrastriatal areas such as the substantia nigra reticulata and thalamus. In the cortex ipsilateral to the striatal lesion, Fluoro-Jade-stained neurons were located in layer VI and deep layer V throughout the cingular, frontal, parietal, and agranular insular cortex (Fig. 3A). After PHA-L injections, a dense network of immunolabeled fibers was still observed in the ipsilateral and contralateral striata. A clear decrease in axonal terminal arborizations was again not observed on the lesioned side, and highly ramified terminal arborizations coexisted with coarse fibers and axonal varicosities.

In rats subjected to bilateral striatal lesion (group B2), the distribution of Fluoro-Jade-stained neurons was similar to that observed after unilateral lesion. These neurons were bilaterally located in layer VI and deep layer V, and no appreciable increase in Fluoro-Jade-positive neurons was observed in other cortical layers.

Group C (Subacute Lesions)

The lesioned striatum showed notable shrinkage and there was a clear enlargement of the ipsilateral ventricle. There was a small (10%–15%) and statistically nonsignificant decrease in cortex thickness with respect to controls, attributable to similarly small and nonsignificant reductions in the thickness of layers V and VI (~20% decrease; Fig. 1). Cell counts did not reveal significant changes in cell density in any of the layers. Fluoro-Jade-stained sections showed not only stained neurons but also numerous stained astrocytes among the labeled neurons in deep layer V and layer VI (Fig. 3B). Similarly, labeled astrocytes were observed in other areas showing labeled neurons after acute lesion (e.g. spared striatal areas, substantia nigra reticulata, thalamus). After PHA-L injections marked labeling was observed in the ipsilateral striatum, with numerous positive fibers between capsula interna bundles. However, the density of axonal branches and terminal arborizations was clearly reduced. No significant changes were detected in the contralateral nonlesioned striatum.

Group D (Chronic Lesions)

In cresyl violet-stained sections, the lesioned striatum showed dramatic atrophy, and most of the striatal volume had disappeared as result of shrinkage (Fig. 4A) related to progressive clearance of dead neurons and their dendrites and axons, reduction of the gliotic reaction associated with the initial lesion, and degeneration of afferent fibers (see below). Small spared areas were usually observed along the ventricular border or ventrolaterally along the ventral aspect of the external capsule. The remaining tissue consisted of capsula interna bundles close to each other or separated by thin bridges of non-neuronal striatal tissue. There was a marked decrease in cortex thickness with respect to controls (~35% decrease), largely attributable to reduction in the thickness of layer V (45%–50%) and layer VI (45%–50%) (Figs. 1, 4A, C, D). Cell counts revealed a significant reduction in neuron density only in layer VI (~20%–25% decrease). However, the fact that there were no significant changes in neuron density in layer V, together with the marked shrinkage of this layer (45%–50% decrease in thickness) indicates marked neuron loss. In addition, we observed a marked reduction in the number of large pyramidal neurons in layers V and VI. Sections stained with Fluoro-Jade showed practically no labeling in the lesioned striatal area or in spared striatal areas surrounding the lesion. In the cortex, no Fluoro-Jade-labeled neurons were observed except for a few scattered neurons in some rats. However, 1 rat showed a small cavity and necrosis of the spared striatal region, with intense labeling of this area and a group of positive neurons in the cortex. After injection of PHA-L, a dense network of labeled fibers and...
axonal terminal arborizations was observed in the striatum contralateral to the injected cortex (i.e. the nonlesioned striatum). In the lesioned area of the striatum ipsilateral to the cortical injections, PHA-L-labeled fibers were practically absent from the striatal tissue. However, PHA-L-labeled fibers and terminals were located in small areas of apparently spared striatal tissue observed around the lesion in some rats (Fig. 5A–D).

**Group E (Long-Term Grafts)**

In cresyl violet-stained sections, the “adult” (i.e. approximately 1-yr-old) intrastriatal grafts appeared as neuron-rich masses surrounded by densely packed fiber bundles of the internal capsule (Figs. 4B, 5E). The general morphology and development of the grafts have already been described in detail in previous studies (23, 37). There was a small and nonsignificant reduction in total cortex thickness (~15%) (Figs. 1, 4B, E, F). There was a more marked and a statistically significant decrease (~20%–25%) in the thickness of layer VI, but this decrease was significantly lower than that observed in the nongrafted group (group D, ~45%–50%). There was also a small and nonsignificant decrease in the thickness of layer V (~15%), and this decrease was again significantly lower than that observed in nongrafted rats (group D, ~45%–50%). Counts revealed a small and statistically nonsignificant decrease (15%–20%) in neuron density in layer VI, with no appreciable changes in the other layers. Sections stained with Fluoro-Jade showed practically no labeling in the graft or the host cortex. One transplant with a small necrotic area showed intense positivity in this area and a few stained neurons in the cortex. After PHA-L injections, a rich network of labeled fibers and terminals was observed in the contralateral nonlesioned striatum. In the lesioned striatum surrounding the grafts, numerous PHA-L labeled fibers were densely packed between the fiber bundles of the internal capsule. In addition, a large number of labeled fibers were located surrounding the graft and entering the graft to form terminal-like networks extending among the grafted cells. The density of labeled fibers was higher in the peripheral region of the graft and decreased toward the central area (Fig. 5E).

**Group F (Short-Term Grafts)**

The 3-wk-old grafts were already well developed, and in cresyl violet-stained sections were rather similar to the long-term grafts (group E). However, some immature-like areas (composed of small, dark, densely aggregated cells) were still found, particularly in the central core of the graft. No statistically significant changes were observed in the thickness of the whole cortex (~10% decrease), or of the cortical layers (10% decrease in layers V and VI), and cell counts did not reveal significant changes in neuronal densities (Fig. 1). Fluoro-Jade staining showed positive neurons and astrocytes in the cortex; however, the number of labeled cells was clearly lower (~60% decrease) than in nongrafted rats with subacute lesions (Fig. 3D). After PHA-L injections, PHA-L-labeled fibers were abundant in the lesioned area surrounding the graft, and labeled fibers and terminal-like networks were observed within the graft.

**DISCUSSION**

In the present study we have shown that an excitotoxic lesion of the striatum induces long-term changes in the cortex similar to those observed in HD patients. Cresyl violet-stained sections from rats with chronic lesions revealed marked gross atrophy of the cerebral cortex, mostly due to a reduction of ~45%–50% in the thickness of cortical layers V and VI. As also observed in HD, neuronal counts showed small decreases in cell density which, together with the clear shrinkage in layers VI and V, reveal an important reduction in the absolute number of neurons in these layers. Cortical injections of PHA-L in rats with chronic lesions showed that the loss of neurons in the cortex is accompanied by a dramatic loss of corticostriatal afferents to the lesioned striatum. No significant changes in cortical thickness or cell counts were observed shortly after excitotoxic lesion (i.e. acute and subacute lesions, groups B and C). However, PHA-L immunohistochemistry revealed changes in corticostriatal terminals in these animals, and Fluoro-Jade studies showed lesion-induced labeling in neurons of cortical layer VI and deep layer V. Subsequently, Fluoro-Jade labeled cortical astrocytes became increasingly abundant, and finally Fluoro-Jade labeling practically disappeared by 12 months after the lesion (i.e. in animals showing atrophy and neuronal loss with cresyl violet), presumably because all the affected cells had already disappeared.

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**Fig. 3.** Fluoro-Jade-stained sections showing the cortical area asterisked in Figure 4A (A–C) and a grafted striatum (D). Numerous Fluoro-Jade-stained neurons were located in layer VI and deep layer V one week after ipsilateral striatal lesion (A; acute lesion, group B), while numerous stained astrocytes were observed among the labeled neurons 4 wk post-lesion (B; subacute lesion, group C). However, the number of labeled cells observed 4 wk post-lesion in the same cortical area was clearly lower in rats subjected to intrastriatal grafts (C; short-term grafts, group F). D: These short-term grafts (i.e. 3-wk-old grafts) were already well developed and mostly Fluoro-Jade-negative, and were surrounded by intensely stained striatal tissue. Dashed lines indicate graft-host borders. Scale bars: A–C, 100 μm; D, 500 μm.

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Therefore, our results suggest that the striatal degeneration caused by HD contributes markedly to the cortical atrophy observed in this disease. However, the present data do not rule out that the cortex is also affected as a direct consequence of the disease.

Fluoro-Jade is a sensitive marker of neuronal degeneration induced by a variety of well-characterized neurotoxins, including neuronal retrograde degeneration after deafferentation (32, 33, 38). Fluoro-Jade has a number of advantages over traditional histologic techniques such as hematoxylin and eosin staining and suppressed silver methods: Fluoro-Jade is fast and simple, reliable, suitable for multiple label studies, and archival fading is not noticeable after a long time. The present and previous studies have shown that Fluoro-Jade labels degenerating neurons and their processes regardless of the mechanism by which a neuron dies, and detect cells that degenerate via apoptotic or necrotic mechanisms (32, 33). Interestingly, the present study and other recent studies (38) have shown that Fluoro-Jade also labels activated astrocytes, albeit some time after labeling of neurons. The exact mechanism by which Fluoro-Jade stains degenerating neurons has not yet been clarified. It has been proposed that a degenerating neuron produces a strongly basic molecule with affinity for the strongly acidic Fluoro-Jade (32, 33).

In HD, a major argument against secondary cortical atrophy (i.e. retrograde degeneration after striatal atrophy) has been that the distribution of neuronal loss in the cortex does not match the distribution of neurons projecting to the striatum (2). It has been observed that cortical neurons in layers III, V, and VI project to the striatum (39); in HD, however, the neuronal loss has been reported to be more severe in layer VI than in layer V, and particularly than in layer III. In the present study, we found that the retrograde degenerative changes and neuronal loss occurring after the excitotoxic lesion of the striatum are localized mostly in layer VI and deep layer V, as observed in HD. Therefore it is possible that some of the cortical neurons projecting to the striatum are more vulnerable to retrograde degeneration after the excitotoxic lesion. It has been suggested that neurons projecting to the striatum without sending any major collateral projections to other regions may be particularly affected (7). Since the projections to the contralateral striatum (i.e. bilateral projections) originate almost exclusively from layer III and superficial layer V, while deep layer V and layer VI give rise almost exclusively to unilateral (i.e. ipsilateral) projections (40, 41), our results (i.e. Fluoro-Jade labeling of neurons in deep layer V and layer VI ipsilateral to the lesioned striatum) may suggest that cortical neurons projecting bilaterally are spared. However, a few rats subjected to bilateral striatal lesion did not show significant changes in the distribution of the Fluoro-Jade-labeled neurons (i.e. labeling mostly in deep layer V and layer VI). Corticostriatal neurons also a) show differences in local axon collaterals; b) project to different striatal compartments (i.e. deep-layer-V and layer-VI neurons project principally to the patches, whereas superficial-layer-V, layer-III and layer-II neurons project principally to the matrix (36); and c) differ in a number of other respects (13), including expression of neurotrophins, which might explain their different vulnerability to striatal lesion. Interestingly, a very recent study has shown that intrastral excitotoxic lesions or blockade of retrograde transport in corticostriatal neurons result in upregulation of BDNF in neurons of cortical layers II/III, V and VI a few hours after lesion placement, and that intrastral grafting of a BDNF-secreting cell line prevented the increase in cortical BDNF (42). This suggests that the regulation of neurotrophin expression in the cerebral cortex by striatal neurons is required for trophic support of cortical neurons, and that changes in neurotrophin levels may constitute initial adaptative mechanisms (42, 43). However, changes in neurotrophin levels may not be sufficient for preventing cortical degeneration after large striatal lesions such as those produced in the present study or those observed in late stages of HD. Finally, although neuronal loss in the cortex affects particularly the above mentioned neurons, one must take into account that the damage of these neurons probably induces functional changes “en cascade” in other cortical neurons, extending the cortical dysfunction.

The present experiments revealed a pattern of cortical atrophy similar to that observed in HD. However, we also considered the possibility that this pattern could be related to causes different to retrograde degeneration (i.e. methodological artifacts). First, given that neuronal degeneration was principally observed in the deep cortical...
layers, we considered the possibility of retrograde diffusion of ibotenic acid along the needle tract, and subsequent cortical lesion by a local excitotoxic mechanism. However, Fluoro-Jade labeling was similar to and sometimes more intense in areas very far from the needle track than in areas close to it. Likewise in some animals with small striatal lesions (excluded from the present study), Fluoro-Jade-labeled neurons were found only in areas far from the needle track. Furthermore, Fluoro-Jade labeled neurons were also observed in regions far from the injection site, such as the substantia nigra or thalamus, that are connected with the striatum and show degenerative changes after striatal lesions and in HD (4, 6, 44, 45). However, a number of reasons argue against this possibility: i) the extensive and layer-specific damage observed here; ii) the distribution of Fluoro-Jade labeled neurons (see above); and particularly iii) the selective degeneration of corticostriatal fibers (i.e. PHA-L labeled fibers after ipsilateral PHA-L injection) projecting to the lesioned striatum (group D, chronic lesions), while a dense network of labeled fibers was still observed in the contralateral striatum. A nonspecific cortical damage would lead to degeneration of ipsilateral cortical neurons projecting to the ipsilateral striatum and ipsilateral cortical neurons projecting to the contralateral striatum.

Previous studies have shown that intrastriatal fetal-striatum grafts implanted in acutely lesioned striatum (i.e. 1–4 wk after lesion) are innervated by all major host striatal afferent systems (23, 24, 26, 37) and establish efferent graft-to-host projections (25). In the case of the corticostriatal projection and in agreement with the present results, several studies using retrograde and anterograde tracers have demonstrated host cortical afferents to the grafts (23, 24), while ultrastructural analysis has revealed synaptic contacts with the transplanted striatal neurons (27, 28). Functional studies have shown that striatal grafts may respond to cortical stimulation, indicating the development of functional synaptic contacts (29–31, 46). Such grafts have been shown to partially ameliorate lesion-induced behavioral deficits with respect to lesioned and nongrafted animals (21, 47), which has often been attributed to anatomical integration and to establishment of functional graft-host connectivity. Given that the corticostriatal projection constitutes the main afferent input to the striatum and that dopaminergic and serotonergic afferents exert a regulatory influence over the corticostriatal input, it is reasonable to think that the behavioral improvement observed in the grafted animals and the improvement in motor and cognitive functions observed after transplantation of patients with HD (8–10, 45) is largely due to the effects on the corticostriatal system. In addition, some graft-induced changes have been reverted by corticostriatal deafferentation (46). However, this improvement may be due not only to the partial restoration of basal ganglia circuitry, but also to protection of neurons of these circuits against anterograde and retrograde degeneration, probably by providing new trophic support to vulnerable cortical neurons (see above). The present results demonstrate that grafts protect against the retrograde degeneration of cortical neurons by showing a) that 12 months after the lesion, PHA-L-labeled corticostriatal fibers practically disappear from the striatal lesioned area but not in both lesioned and grafted rats, which show a large number of corticostriatal fibers surrounding and innervating the graft; b) that the lesion-induced cortical atrophy was markedly reduced by grafts implanted shortly after the striatal lesion; and c) that there is an early protective effect of the graft on corticostriatal neurons, as revealed by Fluoro-Jade staining. The present data are in agreement with our previous observation of poor cortical innervation of implants placed in long-term lesioned striatum (48), and indicate that the striatal grafts should be implanted in early stages of HD to reduce the degeneration of the corticostriatal system and the extension of the cortical dysfunction.

In conclusion, striatal atrophy leads to long-term cortical atrophy (particularly in layers V and VI), together with a pronounced loss of corticostriatal afferents. Both effects can be markedly reduced by implantation of intrastriatal fetal-striatum grafts shortly after the lesion, suggesting that the behavioral improvement observed in the grafted animals and patients may be due not only to the partial restoration of basal ganglia circuitry, but also to protection of neurons of these circuits against retrograde and anterograde degeneration.

**Fig. 5.** Microphotographs of the striatum of rats killed 1 yr after unilateral striatal lesion and subjected to intracortical injection of PHA-L 10–14 days before being killed. A, B: A dense network of labeled fibers was observed in the contralateral (i.e. nonlesioned) striatum (A), while practically no labeled fibers were observed in the same area of the ipsilateral side of this section (B) (i.e. the center of the striatal lesion; asterisks indicate the same zone). C, D: Striatum contralateral to the PHA-L injections (i.e. nonlesioned striatum, C), showing a dense network of labeled corticostriatal fibers, while in the same region of the ipsilateral side of the same section (i.e. lesioned striatum, D) the labeled terminals had practically disappeared; coarse fibers of passage were labeled, however, in myelinated capsula interna bundles (arrow). E: Intrastriatal graft implanted 1 wk after the striatal lesion (group E), showing dense cortical innervation. The dashed lines indicate the graft-host borders. Scale bars: A–D, 200 μm, E, 300 μm.
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