Striatal neural grafting improves cortical metabolism in Huntington’s disease patients

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Summary
Huntington’s disease is a hereditary disease in which degeneration of neurons in the striatum leads to motor and cognitive deficits. Foetal striatal allografts reverse these deficits in phenotypic models of Huntington’s disease developed in primates. A recent open-label pilot study has shown some clinical improvement or stabilization in three out of five Huntington’s disease patients who received bilateral striatal grafts of foetal neurons. We show here that the clinical changes in these three patients were associated with a reduction of the striatal and cortical hypometabolism, demonstrating that grafts were able to restore the function of striato-cortical loops. Conversely, in the two patients not improved by the grafts, striatal and cortical hypometabolism progressed over the 2-year follow-up. Finally, detailed anatomical-functional analysis of the grafted striata, enabled by the 3D fusion of MRI and metabolic images, revealed considerable heterogeneity in the anatomic and metabolic profiles of grafted tissue, both within and between Huntington’s disease patients. Our results demonstrate the usefulness of PET measurements of brain glucose metabolism in understanding the effects of foetal grafts in patients with Huntington’s disease.

Keywords: cortex; foetal graft; Huntington’s disease; metabolism; striatum

Abbreviations: CMRGlu = absolute glucose metabolic rate; SPM = statistical parametric mapping

Introduction
Huntington’s disease is a dominant autosomal neurodegenerative disease beginning during adulthood and leading to dementia and death within 20 years. To date, no curative nor neuroprotective therapies are effective to arrest the progression of this disease. Convergent neuropsychological, imaging and pathological studies have shown that the degenerative process in Huntington’s disease affects first and foremost the striatum, and more specifically the Golgi type II (‘medium-spiny’) neurons (Peschanski et al., 1995; Cattaneo et al., 2001). This striatal neurodegeneration is revealed by the progressive atrophy of the caudate and putamen, and by a decrease of striatal metabolism that precedes symptom onset in gene carriers (Mazziotta et al., 1987; Grafton et al., 1992; Antonini et al., 1996). At more advanced stages of Huntington’s disease, the striatal hypometabolism progresses and is associated with a decrease in cortical metabolism. This cortical hypometabolism has been attributed to the dysfunction of the cortico-striato-thalamo-cortical loops induced by the striatal disease (Young et al., 1986; Berent et al., 1988; Kuwert et al., 1990; Kremer et al., 1999), but might also be related to the delayed degeneration of cortical neurons associated with the disease process.
et al. motor and cognitive improvement (Kendall 1988; Sirinathsinghi et al., 1998). Our group recently reported the results of an open-label pilot study in which five Huntington's disease patients were grafted with striatal neuroblasts (Bachoud-Leïvi et al., 2000b), showing that three of the patients had some clinical improvement after the graft. Here, we report the results of follow-up of striatal and cortical metabolism in these five patients.

Subjects and methods

Subjects

Ethical permission was obtained from the French National Ethics Committee and the Créteil University Hospital Ethics Committee, and written informed consent was given by all subjects. The follow-up protocol was based upon the full Core Assessment Program for Intracerebral Transplantation in Huntington’s disease (Quinn et al., 1996).

Clinical evaluation

Five patients (mean age 43.4 ± 7.3 years) with clinical symptoms for 2–7 years and genetically identified Huntington’s disease were included (Table 1) (Bachoud-Lévi et al., 2000b). In brief, all patients had choreic movements, dystonia, gait disturbances, cognitive deficits and psychiatric symptoms (irritability, depression). MRI revealed various degrees of atrophy of striatal nuclei. The patients had a 2-year clinical follow-up before the first graft in the right striatum (T0). Twelve months later, they were grafted in the left striatum (T1), and the final evaluation took place 1 year after this second graft (T2). Each of these yearly evaluations comprised a large battery of neuropsychological, motor and psychiatric tests detailed previously (Bachoud-Lévi et al., 2000a, b).

Transplantation procedure

Small blocks of tissue were obtained from the whole ganglionic eminence of fetuses 7.5–9 weeks post-conception (Bachoud-Lévi et al., 2000b). The tissue was stereotactically grafted in the caudate and putamen using a needle with a 0.6 mm internal diameter (Bachoud-Lévi et al., 2000a). Two needle tracks aimed at the head of the caudate and three at the putamen, mainly precommissural. However, some of these tracks could not be performed in several patients because of marked striatal atrophy (Bachoud-Lévi et al., 2000a). Immunosuppression with cyclosporine was maintained for 18 months (6 months after the second graft).

MRI and PET scanning procedures

MRI consisted of a T1-weighted SPGR (spoiled gradient recalled acquisition in the steady state) with inversion-recovery acquisition allowing a 3D reconstruction with 1.2-mm thick axial slices. The PET examination was performed with a high-resolution EXACT HR+ tomograph (CTI/Siemens) using a 3D acquisition, before transplantation and at T2. Twenty minutes prior to PET examination, we inserted a radial arterial line under local anesthesia to allow blood measurements of radioactivity and glucose concentrations. The subject was positioned in the tomograph with the head maintained using an individually molded headholder. The room was quiet and light dimmed during the whole examination. Metabolic images were acquired 30–50 min after intravenous injection of 118–280 MBq of $^{18}$F FDG (fluorodeoxyglucose), and the absolute glucose metabolic rate (CMRGlu) was calculated using the classical model proposed by Sokoloff and revised for human PET studies (Fontaine et al., 1999).

Image analysis

First, we used the statistical parametric mapping (SPM) software (SPM99; Wellcome Department of Cognitive Neurology, London, UK) (Friston, 1996) to perform a voxel-by-voxel t-test comparison of pre- and post-graft brain metabolism of each patient with the brain metabolism obtained in 17 age-matched controls (mean age 42.0 ± 8.1 years). In order to have enough power for the statistical analysis and to avoid any bias, we compared the pre- and post-graft scans of each patient with the same normal database obtained in 17 controls to ensure that the changes between both comparisons could only be related to changes that occurred in the patients, rather than changes in the normal controls dataset. For that purpose, individual PET images were transformed to fit the Talairach’s standard stereotaxic space (Talairach and Tournoux, 1988). The images were then smoothed with a $8 \times 8 \times 8$ mm Gaussian filter to compensate for intersubject variability of brain anatomy (Friston, 1996). The statistical threshold of each comparison was set at a conservative value of $P < 0.0005$, with an extent threshold >20 voxels (1 voxel = 8 mm$^3$). We then counted the number of voxels in which the metabolism was significantly lower than in controls at this statistical level, both in the striatum and in the cortex. These numbers were compared individually at baseline (T0) and at the end of the study (T2).

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**Table 1 Patients’ characteristics at baseline (T0)**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)/sex</th>
<th>Handedness</th>
<th>CAG repeats (n)</th>
<th>Disease duration (years)</th>
<th>TFC</th>
<th>MDRS</th>
</tr>
</thead>
<tbody>
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<td>11</td>
<td>130</td>
</tr>
<tr>
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<td>43</td>
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<td>12</td>
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<td>41</td>
<td>6</td>
<td>12</td>
<td>141</td>
</tr>
<tr>
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<td>38/M</td>
<td>Right</td>
<td>51</td>
<td>9</td>
<td>6</td>
<td>113</td>
</tr>
<tr>
<td>5</td>
<td>48/F</td>
<td>Right</td>
<td>44</td>
<td>4</td>
<td>11</td>
<td>137</td>
</tr>
</tbody>
</table>

TFC = total functional capacity score (Shoulson, 1981); MDRS = Mattis dementia rating scale score; M = male; F = female.
In addition, we quantified the striatal glucose consumption (CMRGlu) in each patient before and after the graft, using individual regions of interest (ROIs). For that purpose, metabolic images were coregistered with the individual MRI obtained at the same time-point (Mangin et al., 1994). Anatomic ROIs delineating the head of the caudate nucleus and the putamen in each hemisphere were drawn on individual MRIs, on all slices where these structures were visible. We copied all ROIs onto corresponding PET images and calculated CMRGlu values in each striatal structure.

Finally, we performed a qualitative analysis of the metabolic profile within the striatum of each subject using a 3D superimposition of metabolic images onto the corresponding MR images. The striatal structures and grafts were also extracted using an MRI segmentation of the brain of patient 1 to better individualize the metabolic profile of both the grafts and the surrounding putamen.

Results
Clinical results
The clinical results have been detailed elsewhere (Bachoud-Lévi et al., 2000b). In brief, patients 1 and 2 were improved by the grafts on global cognitive abilities, tests of executive functions, verbal fluency and motor performances. In patient 3, whose main performances were in the normal range at baseline, a slight decline was observed in some tests of global cognitive abilities or executive functions, but other tests, such as verbal fluency, improved, and he was able to perform again daily activities that he previously gave up. Conversely, patients 4 and 5 did not benefit from the grafts and performed markedly worse at the end of follow-up in nearly all tests of cognitive and motor functions. Clinical outcome was not clearly related to the degree of striatal atrophy measured at baseline, though patient 4, who had the more advanced disease, had a more severe atrophy than the other patients. The whole striatal volume (left + right) was 4412, 5354, 6759, 2777 and 6504 mm³ in patients 1–5, respectively. Accordingly, we were not able to identify any relationship between the technical aspects (e.g. tissue preparation, number of needle tracks, etc.) of the grafts that were performed in each patient and the clinical outcome.

Extent of hypometabolism in patients compared with controls in whole-brain SPM analysis
The number of hypometabolic voxels decreased after the graft in the striatum and in the cortex of patients 1 and 2, with most of the cortical improvement occurring in the frontal lobes. In patient 3, the extent of striatal hypometabolism decreased whereas the cortical hypometabolism was stable at a low level. In contrast, the striatal hypometabolism worsened after the grafts in patients 4 and 5, which was associated with a marked spread of the cortical hypometabolism (Fig. 1).

ROI analysis of the striatal CMRGlu
The amplitude of CMRGlu changes in the striatum between T0 and T2 differed markedly among patients (Fig. 2). Compared with baseline values, CMRGlu values averaged over the right and left striatum increased by 69.8, 28.8 and 7.3% after the grafts in patients 1, 2 and 3, respectively. Conversely, the striatal CMRGlu values decreased over these 2 years by 25.6 and 38.1% in patients 4 and 5, respectively. The changes in CMRGlu were highly correlated (r = 0.99, P < 0.0005) with the changes in the number of hypometabolic voxels in the striatum measured using SPM (see above). The global brain rate of glucose consumption increased from T0 to T2 in patients 1, 2 and 3 (from 5.69 to 8.56, 5.19 to 5.75 and 5.75 to 6.38 mmol/min/100 g, respectively), whereas it decreased in patients 4 and 5 (from 4.88 to 4.64 and 5.53 to 3.99 mmol/min/100 g, respectively).

Qualitative analysis of the striatal metabolism
The superimposition of each metabolic image to the corresponding MRI was used to determine the anatomical-functional profiles of the grafted striata (see Fig. 3). In patient 1, two implants grew markedly in the left putamen (Fig. 4). These grafts have absolute metabolic values in the range of the control mean, corresponding to 2.4- and 2.1-fold over the baseline striatal values in this patients for the anterior and posterior grafts, respectively. Other grafts in patient 1 and in the other patients were smaller, and were difficult to discriminate from the host striatum on MRI. Some of these grafts were associated with a focal increase of metabolism in the corresponding structure (patient 1, left caudate and right putamen; patient 2, left caudate and right putamen; patient 3, right putamen; Fig. 3), while others did not prevent a progressive decrease of striatal metabolism (patients 4 and 5; Figures 3 and 5). Finally, one implant formed a hypometabolic cyst in the left putamen of patient 4 (Fig. 3).

Discussion
The follow-up of brain metabolism in Huntington’s disease patients bilaterally grafted with striatal neuroblasts provided two main results. First, the parallel improvement of clinical status, striatal metabolism and cortical metabolism in patients 1–3 demonstrates that foetal grafts in the striatum of Huntington’s disease patients are able to reconstruct the impaired cortico-striato-thalamo-cortical loops. Secondly, striatal grafts show heterogeneous anatomic and metabolic profiles both within and between patients.

After bilateral grafts, patients 1 and 2 were clinically improved, patient 3 was stabilized, and the disease progressed in patients 4 and 5 despite the grafts (Bachoud-Lévi et al., 2000b). These clinical changes perfectly match the changes observed in brain metabolism. In patients 1, 2 and 3 the mean striatal CMRGlu increased after the grafts, respectively, by 69.8, 28.8 and 7.3%. Moreover, the extent of the cortical...
Fig. 1 Topography and extent of the brain hypometabolism in each patient before and after the graft compared with a group of 17 age-matched controls, revealed by the SPM99 analysis at a statistical level of $P < 0.0005$. All visible voxels have a metabolic value lower than control mean minus 4.01 SD, darkest voxels have the highest difference from control values (Z score). The arrows in patients 1 and 2 indicate the improvement of frontal hypometabolism after the graft. The graphs on the right side indicate the number of hypometabolic voxels before (empty columns) and after (filled columns) the graft in the striatum, and in the whole cerebral cortex.
hypometabolism was reduced in patients 1 and 2 and stabilized in patient 3. These changes were symmetrical despite the fact that grafts were performed 1 year apart. These improvements are unlikely to occur by chance considering the amplitude of the metabolic changes in a region where a 14% decrease is expected on average over this time (Kremer et al., 1999). The evolution of cortical hypometabolism in symptomatic Huntington’s disease patients has not been systematically studied, but the parallelism of striatal and cortical changes in these patients is striking, and therefore unlikely to occur by chance. Indeed, in the two patients who were not clinically improved, striatal and cortical hypometabolism worsened. These results support the fact that the clinical benefit provided by the grafts in the first three patients is related to the functional improvement in the impaired striato-cortical loops. Indeed, it is widely accepted that most of the motor, cognitive and emotional symptoms observed in the early stages of Huntington’s disease reflect fronto-striatal dysfunction (Brandt and Butters, 1986; Lawrence et al., 1998; Joel, 2001). In patients 1 and 2, the improvement of frontal hypometabolism after transplantation is associated with the improvement of cognitive functions involving striato-frontal pathways, for example those measured using the verbal fluency task or the trail-making tests (Bachoud-Levi et al., 2000b). This is in line with the results obtained in primate models of Huntington’s disease, in which striatal grafts improve motor and cognitive deficits induced by a striatal lesion (Kendall et al., 1998; Palfi et al., 1998). Finally, it has been shown in animal models that striatal grafts are able to reconnect both the striatal afferents and efferents (for a review see Wictorin, 1992). Bringing together these experimental results and the present study has two major implications. First, it is possible to improve the function of striato-cortical loops by grafting foetal cells in the striatum, which demonstrates that these cells partially replace the degenerated neurons in the local circuitry. Secondly, the cortical hypometabolism is reversible in Huntington’s disease.

Fig. 2 Absolute CMRGluc values (mg/100 g tissue/min) in the whole striatum of the five patients before (empty columns) and after (filled columns) the graft. The plain and dotted lines indicate the normal mean and the normal mean minus 2 SD, respectively.

Fig. 3 Striatal metabolism in five patients with Huntington’s disease, before (T0) and after (T2) bilateral neural graft in the caudate and putamen. In each individual, the normalized metabolic images have been superimposed on the corresponding MRI. The right side of the brain is on the left in the images. In patients 1–3, the arrows indicate grafted tissue in which metabolism increased compared with the baseline striatal metabolism. In patients 4 and 5, none of the graft was associated with a noticeable increase of metabolism. Note that in patient 4, a cystic cavity appeared in the grafted left putamen (arrow).
disease, at least at this early stage of the disease, which means that it is not related only to the degeneration of cortical neurons. Moreover, this specific remote improvement of frontal cortical metabolism after the striatal grafts supports the rationale for intrastriatal grafting in Huntington’s disease (Peschanski et al., 1995).

However, a potential limitation of this experimental treatment is the heterogeneity of clinical and PET changes after the grafts. In their study, Hauser and colleagues found no significant worsening in seven patients after bilateral foetal grafts in the striatum (Hauser et al., 2002). In this group, the mean striatal metabolism did not change significantly (~2%) 1 year after the second graft; however, no individual data have been provided. In our study, despite the consistency of the technique used in the five patients, we found that the foetal tissue implanted in the striatum of Huntington’s disease patients may have variable anatomical–functional profiles even in the same individual, while using a unique suspension of foetal cells. For example, some grafts grew markedly in the host striatum, whereas others were difficult to distinguish from the host striatal tissue. Changes in striatal metabolism were not consistently associated with signal changes on MRI. In addition, in patient 4, who was initially improved by the graft, a cyst developed from the graft in the left putamen. If this cyst was caused by graft rejection, it is important to note that it was not associated with any increase of glucose metabolism. These heterogeneous anatomic and metabolic profiles of the grafts are in line with the post mortem observation of the grafts surviving in a single Huntington’s disease patient reported by Freeman et al. (2000). The explanation of this heterogeneity in graft development and metabolism remains speculative. It might be related to the number of surviving cells and to the proportion of striatal tissue included in each implant, or to the degree of reconnection of striatal grafted tissue with the host systems (Clarke et al., 1988; Dunnett et al., 1988; Sirinathsinghji et al., 1988). The fact remains that the uptake of FDG is mainly related to synaptic activity, and that clinical improvement
was observed only in the patients whose grafts induced an increase in global striatal metabolism.

To conclude, our results demonstrate that cortical hypometabolism in Huntington’s disease can be reversed in some patients, at least at early stages of the disease, and that alleviation of striatal dysfunction is sufficient to trigger such a physiological recovery. These results also support the usefulness of PET measurements of brain glucose metabolism in understanding the effects of foetal grafts in patients with Huntington’s disease.

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