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Human Fetal Striatal Transplantation in Huntington's Disease: A Refinement of the Stereotactic Procedure

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Key Words
Huntington's disease · Fetal tissue grafting · Brain repair · Neurotransplantation · Stereotactic techniques

Abstract

Background: Human fetal striatal transplantation (HFST) is an experimental stereotactic intervention in the treatment of Huntington's disease (HD). This procedure has proved feasible, safe, well tolerated and it offers a potential strategy for brain repair in HD patients. Target areas are the nucleus caudatus caput (NCc) and the precommissural and postcommissural putamen (Pu). A suboptimal spatial distribution of grafts was frequently reported, especially for the postcommissural Pu, because of striatal atrophy and the concurrent ventricular frontal horn enlargement. An improvement of the stereotactic procedure aimed to optimize the intrastriatal placement of grafts is therefore considered a timely issue. ***Methods:*** Eight consecutive HD patients underwent bilateral HFST. For the first 6 procedures (first group) we performed both caudate and putaminal tracks through a single frontal entry point. For the following 10 procedures (second group), we adopted two completely distinct routes, with two separate entry points, for NCc and Pu tracks. The average number of stereotactic tracks and the average infused volume of tissue suspension were compared between the two groups. ***Results:*** The average number of putaminal tracks and the average infused volume of suspension were significantly higher in the second group. ***Conclusion:*** Adopting two separate routes for caudate and putaminal trajectories allowed us to achieve a larger amount of fetal tissue deposits and a better spatial distribution of grafts.

Introduction

Human fetal striatal transplantation (HFST) is an experimental stereotactic intervention in the treatment of Huntington's disease (HD). Feasibility and safety of this procedure have been demonstrated [1–5]. Moreover, in unblinded pilot studies, HFST has been reported to offer long-term clinical improvement or stability [6]. Based on postulata and experimental data, target areas for transplantation are the nucleus caudatus caput (NCc) and the pre- and postcommissural putamen (Pu) [7]. The group with the greatest experience in HD neurotransplantation performed caudate and putaminal grafting through a frontal parasagittal approach [7]. Actually, adequate intrastriatal grafting was reported to be extremely challenging [1] and the same authors advocated an optimization of the neurosurgical technique, in order to improve the therapeutic value of the intervention [6]. Likewise, in our experience it soon emerged that the use of a single stereotactic approach for caudate and putaminal grafting resulted in a limited number of tracks and in a suboptimal spatial distribution of grafts, especially for the posterior Pu. Therefore, in order to achieve the largest

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Table 1. Data on stereotactic procedures in 3 HD patients undergoing HFST with a single entry point approach (first group, upper part) and in 5 HD patients undergoing HFST with a double-entry point approach (second group, lower part)

Procedure	Patient	Side	Striatal atrophy	NCc tracks	Pre-C Pu tracks	Post-C Pu tracks	Grafted suspension volume, µl
1	1	right	2	1	2	1	200
2	2	right	4	2	2	1	250
3	1	left	2	2	2	1	250
4	2	left	4	1	2	0	150
5	3	left	4	2	2	1	250
6	4	right	4	2	2	1	250
Mean			3.3	1.67	2	0.83	225
7	3	right	3	2	3	2	350
8	4	left	4	2	2	2	300
9	5	left	3	2	2	3	350
10	6	right	2	2	3	3	400
11	7	right	4	2	3	3	400
12	7	left	4	2	4	2	400
13	6	left	2	2	3	3	400
14	5	right	4	2	4	3	450
15	8	right	2	2	4	3	450
16	8	left	2	2	5	3	500
Mean			3	2	3.3*	2.7**	360**

Left and right striatal atrophy were evaluated separately by an experienced neuroradiologist using a semiquantitative visual scale. Atrophy score was assessed on T_2-weighted MRI on the coronal section where the NCc area was maximal (4–16 mm anteriorly to the commissura anterior cerebri). This score is based on visual rating of the shape of the lateral wall of the frontal ventricular horn and of the size of the NCc. The score of NCc atrophy was assumed to be an index of global striatal atrophy. 0 = Convex-shaped ventricular wall. NCc is normal-sized. 1 = Convex-shaped ventricular wall. The portion of the NCc protruding into the ventricle retains more than 50% of its normal size. 2 = Convex-shaped ventricular wall. The portion of the NCc protruding into the ventricle is less than 50% its normal size. 3 = Flat ventricular wall. 4 = Concave-shaped ventricular wall. The mean number of pre- and postcommissural putaminal stereotactic tracks and the mean infused volume of suspension differed significantly between the first and the second group. * $p < 0.005$; ** $p < 0.001$. Pre-C = Precommissural; Post-C = postcommissural.

possible amount of deposits and a better spatial distribution in the whole striatum, we devised a double stereotactic approach with one entry point for the NCc trajectories and a second one for those in the Pu. We report on the results obtained with this refined technique, in terms of stereotactic track number, intrastriatal spatial distribution of grafts and infused volume of tissue suspension.

Materials and Methods

Population

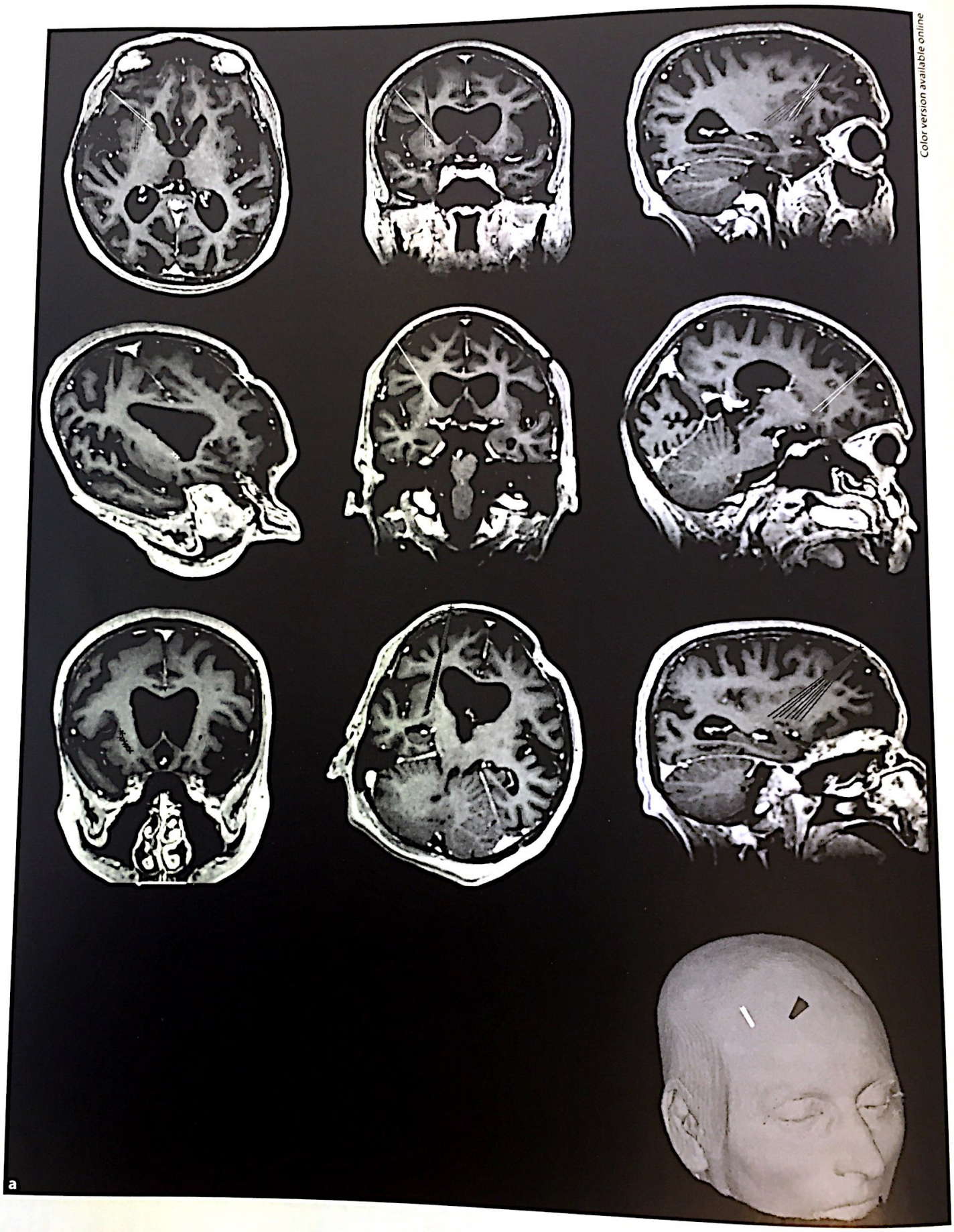
Since February 2006, after the approval of the National Ethics Committee and authorization of the Italian Health Ministry, we performed 16 HFST procedures on 8 clinically and genetically confirmed HD patients (3 females and 5 males, age range: 32–56 years, mean: 45.9 years), who underwent bilateral transplantation in two sessions, 1–5 months apart. Patients presented choreic movements and an individual set of other motor, cognitive and psychiatric symptoms. Magnetic resonance imaging (MRI) showed various degrees of caudate-putaminal atrophy (table 1).

Neurotransplantation Technique

Striatal tissue was obtained from both the whole ganglionic eminences of 9- to 12-week-old legally aborted fetuses. After isolation, tissue was washed twice with saline solution (0.9% w/v of NaCl) and cut into small fragments (about 1–2 mm^3), gently dissociated and resuspended in 500 µl of saline solution (final grafting suspension). 1.5-tesla MRI (Siemens Symphony) using 3-dimensional contrast-enhanced T1 MPRAGE sequence (slice thickness 1 mm, FOV 256 × 256) was performed 1–4 days before each procedure. During MRI examination patients were allowed to breathe spontaneously under mild sedation. Immunosuppression started 1 day before the first procedure (cyclosporin A: 5 mg/kg/day for 12 months; methylprednisolone: 40 mg/day for 3 days after surgery, then tapered within 2 weeks, and azathioprine: 3 mg/kg/day up till now). On the day of surgery, stereotactic computed tomography was used to calculate coordinates after coregistration with the previously acquired MRI examination. Transplantation was performed under general anesthesia, in robotic-assisted conditions (NeuroMate Robotic System, Schaerermayfield, France), within 4–6 h after abortion. For the first 6 procedures we performed both caudate and putaminal tracks through a single frontal entry point. For the following 10 procedures, we adopted two completely distinct routes, with two burr holes, for caudate and putaminal trajectories (fig. 1a). Burr holes were performed with a 6-mm drill. Once the dura was coagulated, a guiding tube (1.2 mm outer diameter) with a hemispheric tip stylet (Alcis, France) was advanced up to the deepest target point of the first track. The stylet was then removed and replaced with a noncoring, beveled needle (0.8 mm outer diameter) connected to a 50-µl Hamilton syringe. At the level of each trajectory, a total of 50 µl of grafting suspension was manually injected into 6–8 sites at 1-mm intervals as guidance tube and transplantation needle were simultaneously withdrawn. For each deposit, once the grafting suspension was injected, the piston of the Hamilton syringe was locked and a rest period of 60 s was allowed to pass before moving up the needle. The same procedure was repeated for each planned track.

Results

Our follow-up ranges from 1 to 22 months (mean: 11 months). In the postoperative period no patient demonstrated adverse effects or clinical deterioration. In all cases MRI performed within 1–4 days after HFST did not



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detect any complication related to surgery and demonstrated the needle tracks and the appropriate location of graft deposits into the target areas (fig. 1b). In 1 patient there was a subdural hematoma necessitating surgical evacuation one month after each procedure, without any neurological consequences. Results concerning the stereotactic procedures are detailed in table 1. Adopting a double approach technique allowed us to perform a significantly higher number of intrastriatal stereotactic tracks, particularly in the postcommissural Pu.

Discussion

The goal of HFST in HD is to substitute lost caudate-putaminal neurons with striatal neuroblasts in order to reconstruct the specific damaged neural circuitries [8, 9]. The surgical technique was adapted by the procedure carried out for intrastriatal grafting of embryonic dopaminergic neurons in Parkinson's disease [10]. Actually, the severe atrophy seen in HD deeply alters the surgical anatomy and poses specific operative challenges. Effective grafting of NCc necessitates a stereotactic route approximately parallel to the major axis of the nucleus, almost tangent to the frontal ventricular horn and passing through the white matter located between the ventricle and the cortical plica linking the gyrus brevis anterior of the insula and the gyrus frontalis inferior. In point of fact, the slope of caudate trajectories is determined by the pattern of nuclear atrophy, by the resulting shape of the enlarged ventricular horn and by the distance between the ventricle and the insular cortex. The more enlarged the ventricular frontal horn is, the smaller will the angle formed by the trajectories and the horizontal plane be, and thus the more lateral and anterior will the corresponding entry point be. In order to graft the Pu in its full thickness, through a frontal approach, reaching the most posterior portion of the still visible nucleus, it is necessary to plan a fan of stereotactic trajectories, passing through the same narrow portion of white matter crossed by the caudate tracks. The narrowness of such an anatomical strait and the direction of the major axis of the nucleus (which, on a horizontal section, shows a comma-like posteromedially concave shape) make obliged the

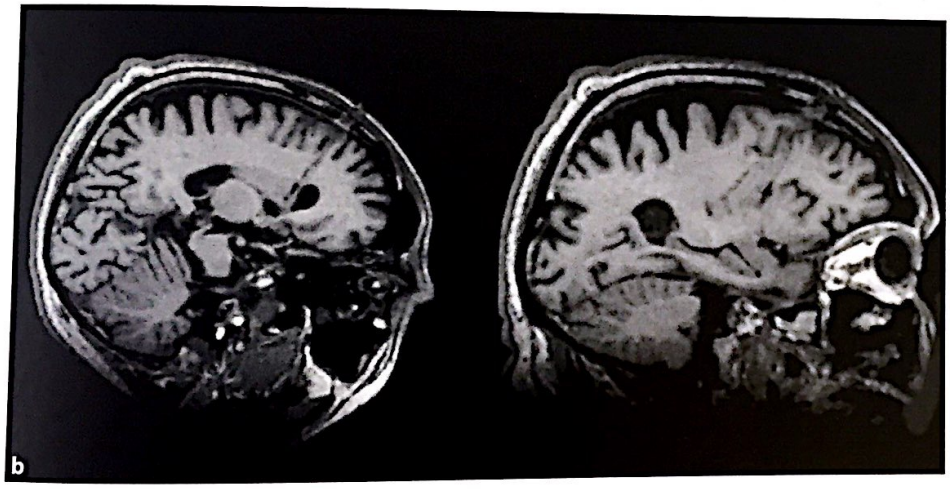

Fig. 1. 3-dimensional magnetic resonance images generated by the neuronavigation workstation from contrast-enhanced T1 MPRAGE sequence. **a** Stereotactic planning of procedure n = 14. First row: projection of NCc (yellow/white) and Pu (red/grey) stereotactic tracks on a multiplanar view; cross points indicate the location of targets. Second row: trioblique view of NCc stereotactic tracks. Third row: trioblique view of Pu stereotactic tracks. Bottom right corner: image of the patient's scalp surface showing the location of the entry points for caudate and putaminal sets of stereotactic tracks. Planning of caudate grafting involves two tracks having a common precoronal point of origin and the inferior extremities located, respectively, in the anterior and posterior portion of NCc, not below the bicommissural plane. These tracks pass through the largest thickness of NCc, without violating the lateral wall of the ventricular frontal horn. Planning of putaminal tracks involves individuation of a point in the lowest and most posterior region of the discernible Pu. Assuming that it is the extremity of the most posterior putaminal track, several target points, 2 mm apart along the major axis of the nucleus, are further chosen on the same plane, distributed in the pre- and postcommissural Pu. The point of origin of such a fan of trajectories is defined so that they will avoid laterally the insula and medially the ventricular horn. **b** Postoperative images obtained 3 days after neurotransplantation. Trioblique view of the NCc tracks (left) and of the fan of Pu tracks (right).

Table 2. Data from the literature concerning the number of caudate and putaminal tracks obtained in HD neurotransplantation

Group	Patients	Procedures	Average of NCc tracks	Average of Pu tracks
Los Angeles [4]	3	6	1	4
Créteil [1]	5	10	1.6	2.8
NEST-UK [5]	4	4	1.75	3.25
Tampa [3]	7	14	1.14	4.5

orientation of this fan of trajectories on the sagittal plane. Definitively, as seen on a verticofrontal plane, optimal caudate trajectories follow a lateromedial direction, while the optimal fan of putaminal tracks follows a parasagittal route. To force into a single point of origin the two sets of trajectories may involve anatomical conflicts between the caudate tracks and the ventricle or between the putaminal trajectories and the insula, according to the position of the entry point. Performing two distinct approaches, with two distinct entry points, allows to completely free each set of stereotactic routes, optimizing it with respect to its specific anatomical constraints. The respective burr holes will be located precoronally, the one for caudate trajectories being more lateral than the other. Adopting this refined approach, we were able to safely perform, even in patients exhibiting major striatal atrophy, a higher average number of caudate and putaminal tracks as compared with our own experience with the single approach method and with data reported by other groups (tables 1, 2). A better spatial distribution in the whole Pu was also achieved, as shown by the comparison between results obtained with the single entry point technique and those obtained using a double-entry point (table 1). Furthermore, our technical refinement theoretically reduces the risk of ventricular rupture, thus limiting intraoperative brain shift, which is potentially responsible for anatomical inaccuracy of grafting.

Conclusions

Adequate caudate-putaminal transplantation is a crucial point to increase the chances of success of HFST in HD. The amount and the intrastriatal spatial distribution of grafted tissue are directly correlated to the degree of caudate and putaminal atrophy. During the planning phase some risks of targeting inaccuracy exist due to the difficulty in individuating on imaging the boundaries of the atrophic striatum and due to the impossibility of matching the patient's brain anatomy with normalized reference system-based stereotactic atlases. Moreover, the risk of brain shifting, and thus of poorly located grafts, is higher in HD because of the profuse intraoperative CSF loss due to cortical atrophy. These issues make HD neurotransplantation challenging. The higher number of stereotactic trajectories may increase the risk of intraparenchymal hemorrhages, but this has never been observed in our experience.

The clinical impact of our technical refinement cannot be assessed at this follow-up. In fact, clinical benefits require at least 2–3 years to manifest, the time needed for graft to develop, connect and restore function [6, 11]. Nevertheless, our operative nuance, at best allowing to tailor the stereotactic procedure to the patient's anatomy, provided an optimization of the neurotransplantation technique in HD, such a devastating disease.

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