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Research Note

Behavioural effects of human fetal dopamine neurons grafted in a rat model of Parkinson's disease*

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Summary. The ventral mesencephalon, containing the developing dopaminergic neurons of the substantia nigra-ventral tegmental region, was obtained from aborted human fetuses of 9–19 weeks of gestation. The tissue was grafted into the striatum of rats previously subjected to a 6-hydroxydopamine lesion of the mesostriatal dopamine pathway. The graft recipients were immunosuppressed by daily injections of Cyclosporin A. Amphetamine-induced motor asymmetry was reduced, and finally totally reversed, only in rats receiving grafts from the 9-week old fetal donor. The fluorescence microscopic analysis revealed large numbers of surviving dopamine neurons, and extensive fiber outgrowth into the host striatum, in these rats. By contrast, rats receiving grafts from 11–19 week old donors had at most only few surviving dopamine neurons. These results indicate that human fetal mesencephalic tissue may be an efficient source of dopamine neurons for functional intracerebral grafting in patients with Parkinson's disease.

Key words: Neural transplantation – Human fetus – Dopamine – Cyclosporin A – Parkinson's disease

Introduction

In Parkinson's disease (PD) the progressive degeneration of mesostriatal dopamine (DA) neurons eventually leads to motor symptoms, primarily hypokinesia, rigidity and tremor. Despite the marked symptomatic relief provided by levodopa treatment, a large proportion of PD patients gradually become severely disabled within a few years after the onset of the disease. A novel strategy for the treatment of PD has been stimulated by the successful functional grafting of catecholamine-producing tissues to the striatum in animal models of the disease (Brundin and Björklund 1987; Olson et al. 1985). So far 4 PD patients have received intrastriatal implants of chromaffin cells obtained from their own adrenal medulla (Backlund et al. 1985; Lindvall et al. 1987, submitted). However, the clinical effects of these grafts have only been minor and transient (Lindvall et al. 1987, submitted). Therefore it is highly warranted to seek other sources of donor tissue that can be used for implantation into DA deficient regions in the Parkinsonian brain. Fetal DA neurons from rodents and, more recently, primates, have been shown to survive intracerebral transplantation to the striatum of DA depleted recipients and give rise to more pronounced and long-lasting functional effects than adrenal medullary tissue (for review see Brundin and Björklund 1987; Olson et al. 1985). If human fetal DA neurons are to be used for grafting in PD patients, however, it is important to establish optimal dissection, parameters of preparation, and donor-age limitations. Cross-species transplants of mesencephalic tissue can consistently yield functional DA grafts in rats, if the host is immunosuppressed with Cyclosporin A (Brundin et al. 1985b). This suggests that the immunosuppressed rat can provide a good model to explore the utility of human fetal mesencephalon as a source for DA neurons in clinical grafting.

The present study was undertaken to clarify to what extent DA neurons from human fetuses of different developmental stages can survive intracerebral grafting, reinnervate the host striatum and compensate for motor abnormalities in rats with lesions of the mesostriatal DA pathway.

* A preliminary account of some of the results from this study was presented at the New York Academy of Sciences meeting on "Cell and tissue transplantation into the adult brain", New York, April 4, 1986

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Material and methods

Lesion surgery and behavioural testing

Female Sprague-Dawley rats (ALAB, Stockholm, Sweden), weighing 180–200 g, were given 2 unilateral stereotaxic injections of 6-hydroxydopamine (6-OHDA, 3 μg/μl in 0.2 mg/ml ascorbate-saline) into the right ascending mesotelencephalic pathway. Briefly, 2.5 μl were injected into the mesostriatal DA pathway (Björklund et al. 1980), and 2 μl into the DA fibers originating from more medial VTA cells (Dunnett et al. 1984). Two to three weeks after the lesion the rats were given 5 mg/kg of dexamphetamine i.p. and their rotational behaviour was monitored in automated "rotometer bowls" for 90 min (Ungerstedt and Arbuthnott 1970). Twenty-six rats that exhibited a mean of at least 5.6 full body turns per min ipsilateral to the lesion were selected for transplantation. An additional 4 rats were left as ungrafted lesion controls. Six rats that did not reach the rotation criterion for complete DA denervation of the striatum were used as graft recipients in the PC–16a group (see below). All rats with complete DA denervation were tested again 2–5 times for amphetamine-induced rotation at various times after transplantation (see Fig. 2A).

Donor tissue, experimental design and drug treatment

Six groups of rats with 6-OHDA lesions received implants of mesencephalic tissue from aborted human fetuses of different post-conceptional ages (time from fertilization), between 1 and 5.5 months after the lesion. The fetuses were obtained at induced abortions[1] and neural grafting was then performed within 2–5 h. Four fetuses, aged 15, 16, 16 and 19 weeks (denoted PC-15, PC-16a, PC-16b, PC-19), were obtained by hysterotomi, and 2 fetuses, 9 and 11 weeks old (PC-9 and PC-11), by careful removal from the uterus using forceps prior to a suction curretage.

Two modes of graft preparation were used (number of hosts reaching final histological analysis is given in brackets). In the PC-9 (n = 7), PC-11 (n = 6), PC-15 (n = 5) and PC-16a (n = 6) groups the region of the ventral mesencephalon containing DA neurons was dissected (as shown for PC-9 in Fig. 1A, B) and prepared according to the cell suspension method (see Brundin et al. 1985a – the PC-9 donor tissue was dissociated in about a 40 μl volume); 4μl of the cell suspension was implanted into each host. In the PC-16b (n = 5) and PC-19 (n = 3) groups the DA-neuron-containing mesencephalic regions were cut into small (approx. 0.5 mm^3) pieces in glucose-saline and injected (2–2.5 mm^3 per host) using a 10 μl glass capillary attached to a 25 μl Hamilton syringe. All graft injections were made into the head of the caudate-putamen (A: +0.9 mm; L: 2.8 mm; V: 5.0 and 4.1 mm; with reference to bregma and dura respectively and with the toothbar set at zero).

Grafted rats were given 10 mg/kg i.p. of Cyclosporin A daily (Sandimmune®, Sandoz, 50 mg/ml diluted to 10 mg/kg in sterile saline) starting either on the day of transplantation or the day before. All the animals were administered tetracyclin (approx. 20–50 mg/kg daily) through the drinking water.

Catecholamine histofluorescence

Five to 20 weeks after transplantation, the brains were processed for catecholamine histofluorescence according to the ALFA method (Lorén et al. 1980; procedure I). Catecholamine cell body counts in the grafts were performed on every third section (15 μm thickness) and the cell number estimated according to the formula of Abercrombie (1946).

1 Permission to conduct this study was granted by the research ethical committee at the Medical Faculty of the University of Lund

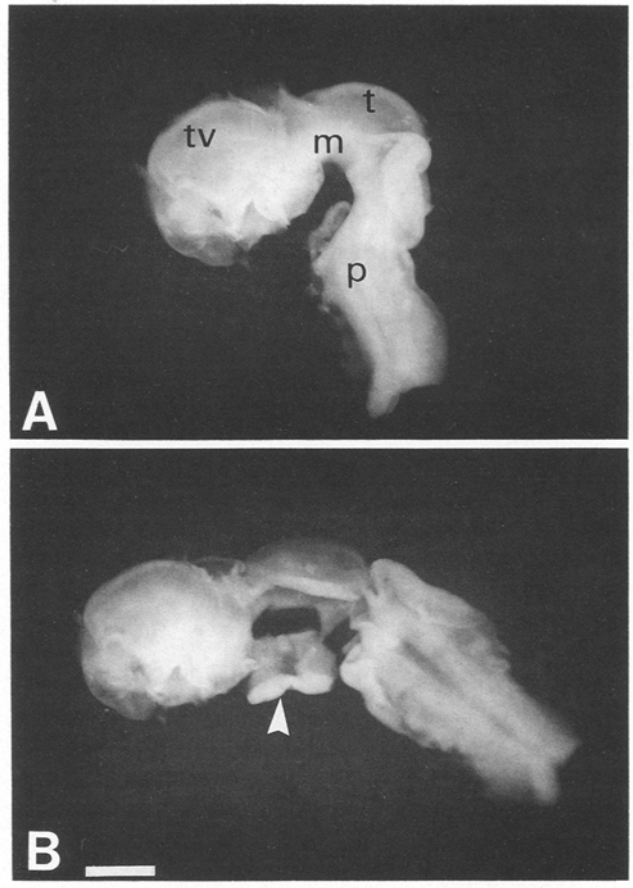
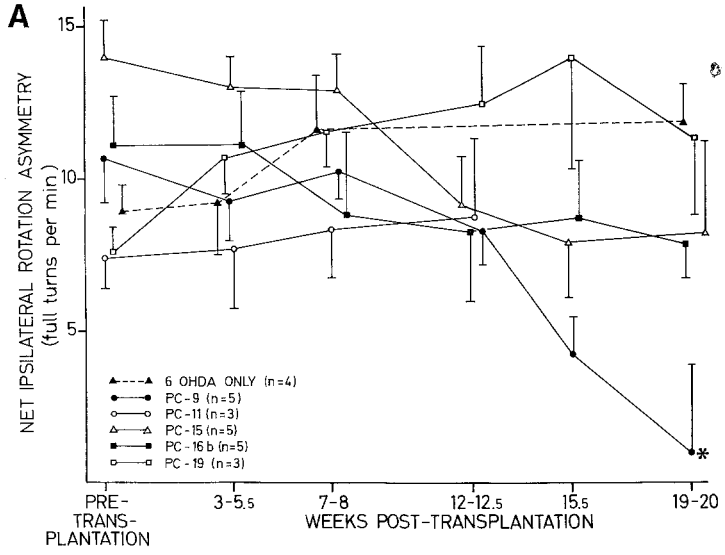


Fig. 1. A The brain and rostral spinal cord, in a lateral view, of the 9-week old fetus used in the PC-9 group. tv = telencephalic vesicle; m = mesencephalic flexure; t = tectal primordium; p = pontine flexure. **B** Ventral-lateral view of the same fetal brain after the ventral mesencephalic piece used for grafting (arrowhead) had been dissected. Scale bar = 2 mm

Results

Motor asymmetry

The group means for amphetamine-induced rotational asymmetry are summarized in Fig. 2A for the 6 groups of rats tested. The 4 grafted groups tested up to 19–20 weeks after transplantation responded differently to amphetamine administration during the course of the experiment (two-way analysis of variance (ANOVA) with repeated measures; group x time interaction; $F(15,28) = 2.55$; $p < 0.02$). This interaction was due primarily to a significant reduc-



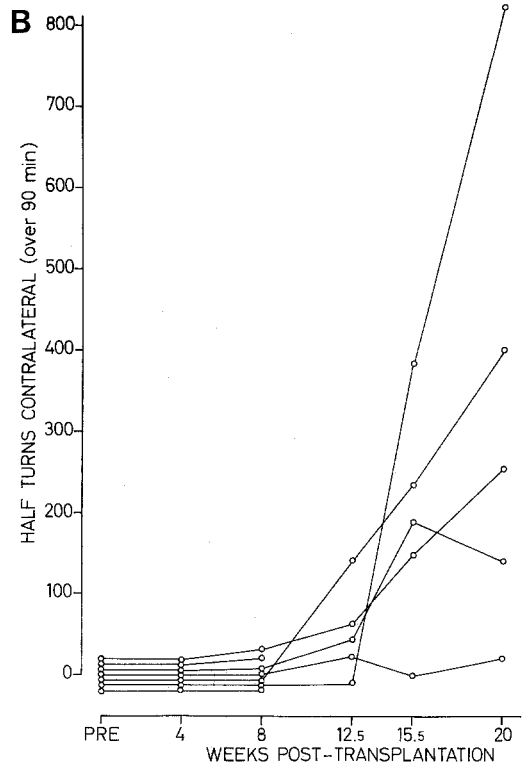


Fig. 2. A Motor asymmetry (mean ±SEM of net 360° turns/min over 90 min in the direction towards the lesion side) induced by 5 mg/kg d-amphetamine, before transplantation and at different timepoints after transplantation. For each group only the rats which survived throughout the test period have been included. * = different from 6-OHDA alone at $p < 0.05$ (ANOVA followed by Newman-Keuls' test). **B** Total half turns displayed in the direction *away* from the lesioned and transplanted side for the individual rats in the PC-9 group. Note that 2 of the rats were perfused for fluorescence histochemistry at 10 weeks post-transplantation

tion in turning in the PC-9 group (repeated measures ANOVA $F(5,13) = 11.61$; $p < 0.001$). In the 19–20 week test only the PC-9 group showed significantly less turning than the 6-OHDA group (ANOVA $F(4,17) = 3.28$; $p < 0.05$ post-hoc Newman-Keuls' test).

In the PC-9 group, 4 of the 5 rats that were tested 20 weeks after transplantation showed a reversal in net rotational asymmetry, with more turns contralateral than ipsilateral to lesion. Clear signs of the behavioural compensation in these rats first appeared at 15.5 weeks after transplantation when 3 of the 4 rats showed between a 69% and 92% reduction in rotational asymmetry. In 3 of the 4 rats that eventually exhibited compensatory changes the ability to turn contralateral to the lesion appeared already at 12.5 weeks (Fig. 2B).

Morphological analysis

Transplant survival. In the PC-9 group two rats were perfused 10 weeks after transplantation. Both showed large surviving grafts with approx. 1400 DA neurons. Four of the 5 remaining rats, perfused after 20–21 weeks, exhibited large grafts with between 300 and 1800 surviving DA neurons (mean = 1200). Since the amount grafted to each rat was equivalent to about 1/10 of the dissected piece, these cell numbers indicate that the survival rate of the DA neurons was in the same order of magnitude as in grafts of rat mesencephalic tissue (Brundin et al. 1985a). The numbers are, however, likely to be an underestimation as the DA neurons were often arranged in dense clusters, or intermingled with aggregates of highly fluorescent DA fibers, and therefore difficult to count. The fifth rat, which showed no behavioural recovery, only had 107 DA neurons, and pathological changes, presumably due to infection, were found in the striatum.

In the PC-11 group (perfused after 6–12.5 weeks) all six recipients showed small grafts with the number of surviving DA neurons varying between 14 and 74 (mean = 44).

In the PC-15 and PC-16a groups (perfused after 20 weeks and 5 weeks, respectively) there was no

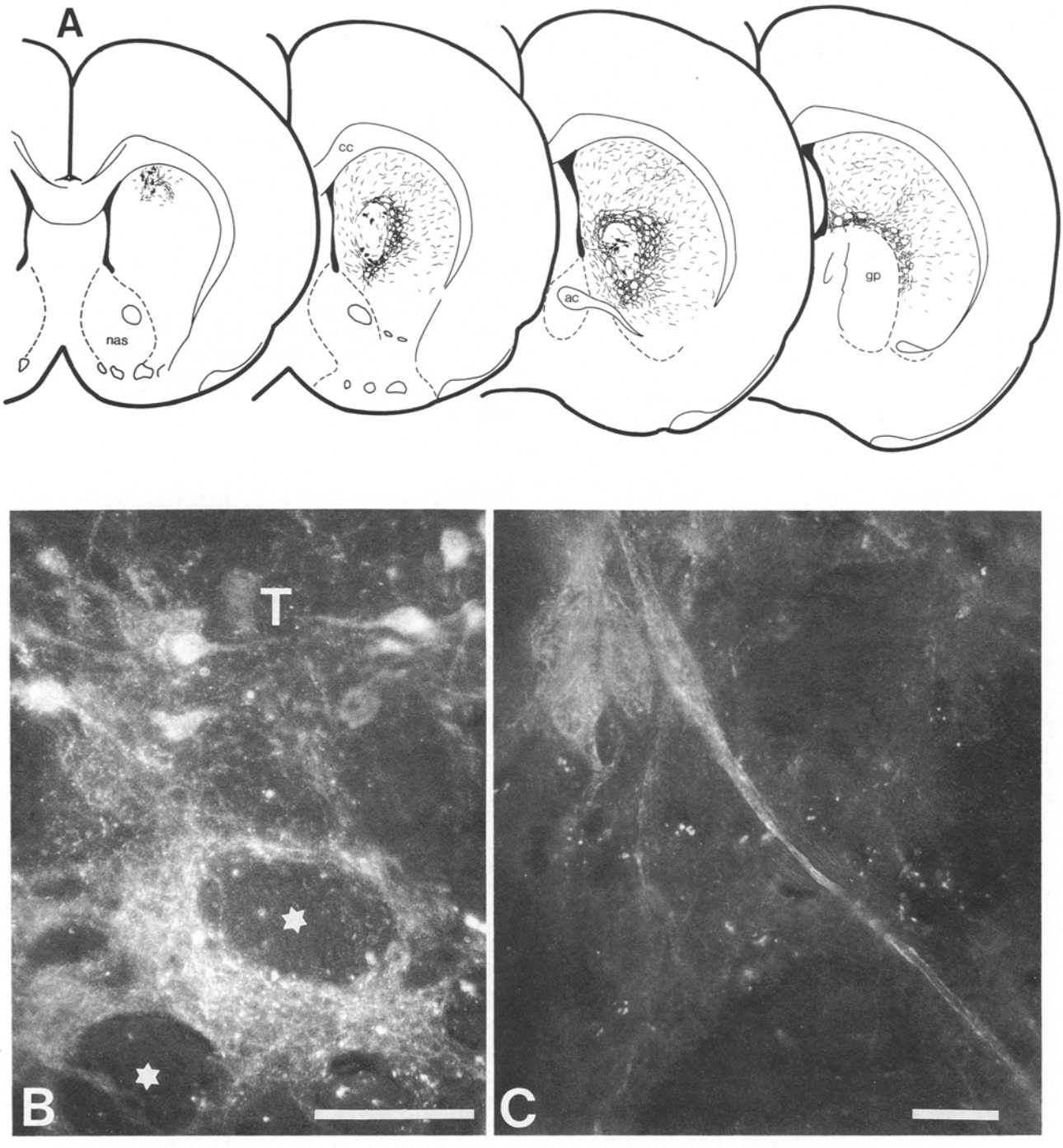


Fig. 3. A Semischematic drawing of a graft from the PC-9 group at 4 different rostrocaudal levels, after 20 weeks survival. The drawings illustrate the intrastriatal graft and its associated DA fiber outgrowth. A total of about 1200 surviving DA-containing neurons was detected in this graft, and the rat was slightly overcompensated in the 19–20 week rotation test. The 6-OHDA lesion of the intrinsic mesostriatal DA pathway was complete, except for some sparing in the olfactory tubercle which is not indicated in the figure. ac = anterior commissure; cc = corpus callosum; gp = globus pallidus; nas = nucleus accumbens septi. **B** DA-containing neurons and associated fiber outgrowth extending into the host striatum in a graft from the PC-9 group. T = transplant; stars = fiber bundles of the host internal capsule. Scale bar = 100 μm. **C** Cluster of DA-containing neurons in a graft from the PC-9 group (containing a total of about 1800 surviving DA neurons). Note the long coarse (probably dendritic) process, characteristic for many of these cells. Scale bar = 20 μm

evidence of surviving grafts and the cannulae tracts were only discernable by a small accumulations of orange fluorescent macrophages.

In the PC-16b and PC-19 groups (both perfused after 19–20 weeks), graft tissue could be identified in 6 out of 8 rats, but surviving DA neurons were found only in 2 of them (one rat in each group) and their number was small (about 30 neurons in each).

Characteristics of grafted DA neurons. In the PC-9 group the DA neurons showed fiber outgrowth into the surrounding host striatum (Fig. 3A, B). As illustrated in Fig. 3A the highest DA fiber density was observed near the graft and distally the axonal network gradually became more sparse. In some cases there was a low density of DA fibers, presumably of graft origin, also in the most dorsolateral portion of the striatum, some 2–3 mm away from the graft. The human grafts formed a dense DA fiber network in the striatal grey matter which was at least as extensive as that of rodent DA neurons. However, compared to DA neurons in syngeneic rat grafts the human DA cell bodies exhibited some distinguishing morphological characteristics. First, a large proportion had a bipolar appearance and very long coarse cell processes (presumably dendrites) (Fig. 3C). Second, a subpopulation of the DA neurons were larger than those found in grafts of rat tissue (Jaeger 1985), and in extreme cases they measured 50 μm along their major axis. This size corresponds to the size of melanin-bearing substantia nigra neurons in the adult human brain (Bazelon et al. 1967).

Discussion

Grafted DA neurons are known to form synaptic connections with the host brain, to be spontaneously active and release DA, and to reverse different kinds of behavioural deficits following damage to the host's own DA system (for reviews see Brundin and Björklund 1987; Dunnett et al. 1985; Olson et al. 1985). This study clearly demonstrates for the first time that also human fetal DA neurons can survive grafting, and give rise to axonal processes that form a new DA terminal network in large parts of the rat striatum, and that they can produce behavioural effects.

In the successful PC-9 group the first effects in the rotation test appeared after 12.5 weeks and were clearly evident by 15 weeks. This is much later than with similar transplants utilizing rodent donors where functional effects can be observed already by 2–4 weeks after grafting (Brundin et al. 1985a, b). Presumably the slower development of functional effects when using a 9 week old human donor is related to the slower time course of the normal development of human DA neurons in situ. The ability to perform body turns contralateral to the lesion (Fig. 2B) was found to appear by 12.5 weeks, i.e. before there was an actual reduction in net rotation asymmetry (15–19 weeks). Thus, consistent with previous rodent studies (Brundin et al. 1986; Herman et al. 1985) this parameter seems to be a sensitive early indicator of graft survival and function. Four of 5 rats showed behavioural "overcompensation", which has also been observed using fetal rat grafts. The fifth rat, which showed no signs of behavioural recovery, only contained 107 DA neurons. This is fewer than the number of grafted rat DA neurons required to yield functional effects in the amphetamine-induced rotation test (Brundin et al. 1985a).

Experiments with rodents indicate that, if one uses a dissociated cell suspension transplantation technique, there will be good survival of mesencephalic DA neurons only if they are taken from fetuses at a relatively early stage in development which is approximately equivalent to the time when the DA neurons become post-mitotic (Brundin et al. 1985a). In this study the best survival of grafted DA neurons was found when the donor was 9 weeks post-conception, i.e. in a stage of fetal development when the mesencephalic DA neurons have been described as immature neuroblasts with few axonal processes (Olson et al. 1973). Using a donor a mere 2 weeks older there was some survival of DA neurons although not to the same extent, and donors 15–16 weeks old yielded no surviving DA neurons if the dissociated cell suspension technique was used. However, as known from previous work (Björklund et al. 1980) donor age restrictions are less pronounced when solid pieces of mesencephalon are grafted, and, indeed, when solid pieces were implanted from 16–19 week old donors there was often survival of graft tissue, which in 2 cases also contained low numbers of DA neurons. Since all the rats were immunosuppressed it seems unlikely that the differential survival is due to immunological rejection. On the other hand, as all grafted rats were treated with Cyclosporin A it is not definitely established if immunosuppression is necessary for optimum survival of human DA neurons grafted to the rat brain. However, this seems likely as similar mouse-to-rat grafts require immunosuppression for good survival to occur (Brundin et al. 1985b).

The grafted human DA neurons gave rise to a terminal network in the host striatum at least as extensive as that observed for rat and mouse DA neurons grafted with the same technique to the rat striatum (Björklund et al. 1983; Brundin et al.

1985b). However, the cells were larger and possessed more prominent dendrites than their rodent counterparts, and thus seemed to retain some characteristics that they would have acquired if allowed to develop in situ.

These findings indicate that intracerebral grafting of fetal DA neurons could provide a viable approach to ameliorate neurological symptoms in patients with PD. From this study it seems clear that grafts of human fetal DA neurons have a great functional potential, comparable to that of rodent DA neurons, suggesting that they are suitable for transplantation in PD patients. Before clinical trials with grafting of human fetal tissue can be conducted the ethical aspects will have to be further examined and also a series of technical issues, such as optimal dissection, donor-age limitation and yield of DA neurons, need to be explored in more detail.

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References

Abercrombie M (1946) Estimation of nuclear population from microtome sections. Anat Rec 94: 239–247

Backlund E-O, Granberg P-O, Hamberger B, Knutsson E, Mårtensson A, Sedvall G, Seiger Å, Olson L (1985) Transplantation of adrenal medullary tissue to the striatum in parkinsonism: first clinical trials. J Neurosurg 62: 169–173

Bazelon M, Fenichel GM, Randall J (1967) Studies on neuromelanin. I. A melanin system in the human adult brainstem. Neurology 17: 512–519

Björklund A, Dunnett SB, Stenevi U, Lewis ME, Iversen SD (1980) Reinnervation of the denervated striatum by substantia nigra transplants: functional consequences as revealed by pharmacological and sensorimotor testing. Brain Res 199: 307–333

Björklund A, Stenevi U, Schmidt RH, Dunnett SB, Gage FH (1983) Intracerebral grafting of neuronal cell suspensions. II. Survival and growth of nigral cell suspensions implanted in different brain sites. Acta Physiol Scand (Suppl) 522: 9–18

Brundin P, Isacson O, Björklund A (1985a) Monitoring of cell viability in suspension of embryonic CNS tissue and its use as a criterion for intracerebral graft survival. Brain Res 331: 251–259

Brundin P, Nilsson OG, Gage FH, Björklund A (1985b) Cyclosporin A increases survival of cross-species intrastriatal grafts of embryonic dopamine-contained neurons. Exp Brain Res 60: 204–208

Brundin P, Isacson O, Gage FH, Prochiantz A, Björklund A (1986) The rotating 6-hydroxydopamine lesioned mouse as a model for assessing functional effects of neuronal grafting. Brain Res 366: 346–349

Brundin P, Björklund A (1987) Survival, growth and function of dopaminergic neurons grafted to the brain. In: Seil FJ, Herbert E, Carlson B (eds) Progress in Brain Research Vol 71, Elsevier, pp. 293–308

Dunnett SB, Björklund A, Gage FH, Stenevi U (1985) Transplantation of mesencephalic dopamine neurons to the striatum of adult rats. In: Björklund A, Stenevi U (eds) Neural grafting in the mammalian CNS. Elsevier, Amsterdam, pp 451–469

Dunnett SB, Bunch ST, Gage FH, Björklund A (1984) Dopamine-rich transplants in rats with 6-OHDA lesions of the ventral tegmental area: effects on spontaneous and drug-induced locomotor activity. Behav Brain Res 13: 71–82

Herman J-P, Choulli K, Le Moal M (1985) Hyper-reactivity to amphetamine in rats with dopaminergic grafts. Exp Brain Res 60: 521–526

Jaeger CB (1985) Cytoarchitectonics of substantia nigra grafts: a light and electronmicroscopic study of immunocytochemically identified dopaminergic neurons and fibrous astrocytes. J Comp Neurol 231: 121–135

Lorén I, Björklund A, Falck B, Lindvall O (1980) The aluminum-formaldehyde (ALFA) method for improved visualization of catecholamines and indoleamines. I. A detailed account of methodology for central nervous tissue, using paraffin, cryostat or vibratome sections. J Neurosci Meth 2: 277–300

Olson L, Backlund E-O, Freed WJ, Herrera-Marschitz M, Hoffer B, Seiger Å, Strömberg I (1985) Transplantation of monoamine-producing cell systems in oculo and intracranially: experiments in search of a treatment for Parkinson's disease. Ann NY Acad Sci 457: 105–126

Olson L, Boréus LO, Seiger Å (1973) Histochemical demonstration and mapping of 5-hydroxytryptamine- and catecholamine-containing neurons systems in the fetal brain. Z Anat Entwickl-Gesch 139: 259–282

Ungerstedt U, Arbuthnott GW (1970) Quantitative recording of rotational behavior in rats after 6-hydroxydopamine lesions of the nigrostriatal dopamine system. Brain Res 24: 486–493

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