

Transplantation into the human brain: present status and future possibilities

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Transplantation of brain tissue into the adult mammalian CNS is not a new field of research. A century ago, the *New York Medical Journal* contained an article by W Gilman Thompson¹ entitled "Successful brain grafting". He exchanged large pieces of neocortical tissue between adult cats and dogs and analysed some of the grafts microscopically after survival times of up to 7 weeks. Despite the positive title of his paper, the long-term "grafts" probably consisted only of neuron-free remnants and developing scar tissue. Almost 90 years passed before the first animal experimental data appeared clearly indicating the possible clinical usefulness of the neural grafting technique. In 1979 it was shown^{2,3} that intracerebral grafts of rat fetal dopamine (DA) neurons were able to reduce the symptoms of experimental, 6-hydroxydopamine (6-OHDA)—induced Parkinsonism in rats. This was the first example of neurons, being implanted into the adult mammalian brain, which were able to reverse a functional deficit in an animal model of a human neurological disorder. Early on, of course, these findings kindled a hope that it would be possible to develop a transplantation therapy for patients with Parkinson's disease.

Since then, experimental data obtained in rats have suggested the clinical application of the neural grafting technique also in other disorders. In experimental Huntington's disease, induced by intrastriatal injections of an excitotoxin (kainic acid or ibotenic acid), grafted fetal striatum is anatomically integrated into the host brain⁴ and reduces both motor and cognitive deficits.⁵⁻⁸ In two animal models of dementia (young adult rats with fimbria-fornix transections and cognitively impaired aged rats) grafted fetal cholinergic neurons from the septum-diagonal band region grow into the host hippocampus and ameliorate deficits in memory and learning.⁹⁻¹¹ Furthermore, fetal

noradrenergic neurons from the locus coeruleus region implanted into the hippocampus suppress seizure development in hyperexcitable, previously noradrenaline-depleted rats subjected to electrical kindling^{12,13} (an experimental model of complex partial epilepsy in humans).

However, the transition from the first positive animal experimental data to trials in patients should be approached with caution. From the clinical point of view, research in neural transplantation has by far reached the furthest in Parkinson's disease. Whether or not intracerebral neural grafting will be successful in patients with Parkinson's disease will be of great importance for the future application of this technique in other disease states. Therefore, this review will focus on research in Parkinson's disease and summarises present experience with grafting in patients as well as its animal experimental basis. Finally, some issues will be discussed that are of critical importance in order to establish neural transplantation as a real therapeutic alternative in Parkinson's disease and other neurological disorders.

Grafting experiments in animals—fetal substantia nigra

Most basic experimental studies have been carried out in rats with unilateral, 6-OHDA-induced lesions of the mesostriatal DA system. Such rats exhibit a hemiparkinsonian-like syndrome with the following main characteristics: the animals have an asymmetric posture and display rotational asymmetry towards the lesioned side both spontaneously and in response to amphetamine (which releases DA from the intact mesostriatal system). Apomorphine, which stimulates supersensitive DA receptors in the denervated striatum, causes rotation towards the non-lesioned side. The rats also show sensory inattention towards stimuli applied to the side of the body contralateral to the lesion.

Mesencephalic DA-rich grafts implanted directly into or adjacent to the 6-OHDA denervated striatum in the rat can reduce the lesion-induced motor and sensorimotor asymmetries.^{2,3,14,15} The degree of

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behavioural recovery has been found to be related to the extent of the transplant-derived DA reinnervation of the previously denervated striatum.^{3,16} The graft tissue, which is taken from the ventral mesencephalon of 13 to 15 days old rat fetuses, can be implanted into adult rats using two main procedures: (1) solid pieces can either be put in the lateral ventricle or into premade cortical cavities on top of or lateral to the striatum;^{2,3,16,17} (2) a suspension of cells can be injected stereotaxically directly into the striatum.¹⁸⁻²⁰ The implanted DA neurons grow into the denervated host striatum forming a terminal pattern similar to the intrinsic innervation.¹⁹ They also establish synaptic contacts with host striatal neurons^{21,22} and receive afferent inputs from the host brain.²²⁻²⁴ The grafts are metabolically²⁵⁻³⁰ and physiologically³¹ active; they exhibit transmitter synthesis, normal firing patterns, and spontaneous DA release. In the host striatum, the graft-derived innervation causes post-synaptic dopaminergic binding sites to return to normal density³² and normalises firing rates and drug sensitivity of host striatal neurons.³³

Furthermore, DA neurons implanted into specific subregions of the denervated striatum compensate for specific features of the hemiparkinsonian syndrome in 6-OHDA lesioned rats.^{34,35} Amphetamine-induced rotational asymmetry can be reversed by grafts re-innervating the dorsal caudate-putamen, whereas grafts innervating the ventrolateral part of the striatum ameliorate deficits in sensorimotor attention but have no effects on rotational asymmetry. The functional recovery is only obtained if the DA-rich mesencephalic grafts are placed near the target area, that is, within or directly outside the striatum. Implantation of DA neurons into the substantia nigra, the actual site of cell body loss after the 6-OHDA lesion, or along the trajectory of the nigrostriatal pathway, has not led to the behavioural effects accomplished with grafts localised in the striatum.³⁵ This is most probably due to the inability of the grafted DA neurons to extend their axons over a great distance to the forebrain target region.

The functional effects are clearly dependent on the survival and continuous presence of the graft, since if it is removed or destroyed at any time after transplantation, the deficits immediately reappear.^{16,36,37} The graft-induced recovery from motor asymmetries is specific for DA-rich ventral mesencephalic tissue so that intrastriatal grafts of tissue rich in 5-hydroxytryptamine-producing neurons (from the mesencephalic raphe region) or of tissue appropriate to the target (from the striatum) do not lead to any functional compensation.³⁷

Although several motor and sensorimotor deficits in 6-OHDA lesioned animals can be ameliorated by DA-rich intrastriatal grafts, other behavioural abnor-

malities have proved resistant to graft-induced recovery. These include deficits in hoarding behaviour³⁸ and the aphagia and adipsia seen after bilateral 6-OHDA lesions^{39,40} as well as the impairment in the use of the contralateral forelimb for discrete skilled movements after unilateral lesions of the mesostriatal DA system.⁴¹ It has been proposed (see, for example, ref 41) that the differences in the effects of grafts on different behavioural measures reflect the degree of incorporation of the graft into the host striatal circuitry. In their ectopic striatal location, probably only part of the anatomical and functional connections characteristic of normal mesostriatal DA neurons are re-established by the grafts. The differential sensitivity of different aspects of behaviour to graft-induced recovery in the 6-OHDA lesioned rat could have important clinical correlates. Nigral grafts may be effective for some but not for all symptoms of Parkinson's disease in patients. Modifications of the transplantation procedures or priming of the grafts pharmacologically³⁸ might be required to overcome these limitations.

Successful grafting into the striatum of DA-rich, ventral mesencephalic tissue from fetuses has also been reported in non-human primates with MPTP-induced Parkinsonism. Survival of implanted DA neurons in the caudate nucleus or the putamen has been demonstrated microscopically in rhesus monkeys,⁴²⁻⁴⁴ African green monkeys⁴⁵⁻⁴⁷ and marmosets.⁴⁸ Biochemical data⁴⁷ have indicated that the grafted dopaminergic neurons are able to normalise DA turnover in previously severely DA depleted areas also in non-human primates. Some of the studies have reported a long-lasting reduction of the MPTP-induced motor abnormalities, including hypokinesia, rigidity and tremor.^{43-45,47,49}

Grafting experiments in animals—adrenal medulla

Soon after the initial reports of functional effects of fetal DA neurons in experimental Parkinsonism (see above), alternative sources of catecholamine-producing cells suitable for grafting were also being explored. At that time it seemed unclear whether it would ever be possible, mainly for ethical and immunological reasons, to use human fetal tissue for transplantation in man. Although chromaffin cells from the adult adrenal medulla have by far attracted the greatest interest, animal experiments have suggested that other tissues could also be potentially useful for grafting in Parkinson's disease patients, for example, sympathetic ganglia,^{50,51} carotid body glomus cells⁵² and cell lines like PC12.⁵²⁻⁵⁵

Adrenal medulla cells can be implanted into the lateral ventricle^{56,57} or the DA-depleted striatum of rats or monkeys.⁵⁸⁻⁶¹ The number of surviving chromaffin cells is, however, low both in rodents⁵² and particularly

in monkeys.^{59,62,63} Normal chromaffin cells secrete primarily adrenaline and only low amounts of DA.⁶⁴ In long-term intraventricular adrenal medullary grafts, DA and noradrenaline are the predominant catecholamines,⁶⁵ thus suggesting that transplantation causes a shift in the relative amounts of catecholamines produced by the adrenal medulla. There is almost no fibre outgrowth from the implanted chromaffin cells, which is probably due to inadequate activity of nerve growth factor (NGF) in the adult striatum. Addition of NGF to the graft, preferably via continuous infusion through a dialysis fibre for several weeks, significantly increases cell survival, causes a transformation of many cells towards a more neuronal phenotype and greatly enhances the fibre outgrowth into the host striatum.⁵⁸ Survival of adrenal medullary cells has been demonstrated in the rat for at least 17 months after intrastriatal grafting even without addition of NGF,⁵⁸ but there appears to be a need for a continuous supply of NGF to maintain the graft-derived fibre outgrowth in the host striatum.⁵⁸

The functional capacity of adult adrenal medulla cells implanted either into the striatum or the lateral ventricle has only been demonstrated as an ability to reduce drug-induced rotational asymmetry in hemiparkinson rats or monkeys. In unilaterally 6-OHDA-denervated rats, adrenal chromaffin grafts reduce apomorphine- and possibly also amphetamine-induced turning response.^{52,56,58} The adrenal grafts do not restore contralateral sensorimotor inattention⁶¹ and no effect on spontaneous rotational behaviour (except during the first hours after implantation) has been reported. The reduction of apomorphine-induced rotational asymmetry is stable up to 3 months after grafting but then disappears. However, if NGF is added to the graft through continuous infusion either into the striatum or into the lateral ventricle, the functional effect (as assessed by the apomorphine rotation test) is clearly increased^{58,66} and more long-lasting.⁵⁸ With intrastriatal infusion of NGF in the vicinity of the grafts for 4 weeks after implantation, apomorphine-induced rotational behaviour was significantly reduced also at 6 and 12 months after grafting.⁵⁸ Whether this higher functional potency of adrenal medulla grafts supplied with NGF is due to graft differentiation and neurite outgrowth, as suggested by Strömberg *et al.*,⁵⁸ can however, be questioned on basis of the recent report by Pezzoli *et al.*⁶⁶ They found that NGF plus non-catecholamine-producing tissues (sciatic nerve or fat) were as effective as NGF plus adrenal medulla to reduce apomorphine-induced rotational asymmetry in unilaterally 6-OHDA denervated rats.

The very sparse information reported from experiments with adrenal medulla transplantation in non-human primates is in good agreement with the

data obtained in rodents. Bankiewicz *et al.*⁶³ implanted adrenal medullary tissue into the DA-denervated caudate nucleus of unilaterally MPTP-treated rhesus monkeys. Apomorphine-induced rotational asymmetry was attenuated for 3 months after surgery but by 6 months it had returned to control levels. Whether deficits in spontaneous behaviours can be reduced by adrenal medullary grafts in non-human primates is unclear since both improvement of the use of the affected arm in hemiparkinsonian monkeys^{63,67} as well as no effect on this parameter^{44,49} have been described after surgery.

Grafting experiments in animals—comparison between fetal substantia nigra and adrenal medulla

The available data from basic experimental grafting studies using either ventral mesencephalic tissue or adrenal medulla cells clearly favour the strategy with fetal DA neurons in clinical trials. There seems to be three major advantages: *First*, although both types of cells can improve deficits in rotational behaviour apparent after administration of apomorphine or amphetamine, only nigral cells have been convincingly demonstrated to reduce abnormalities in spontaneous behaviours. These include spontaneous rotational asymmetry, sensory inattention and akinesia in 6-OHDA-lesioned rats and hypokinesia, rigidity and tremor in MPTP-treated monkeys. From the clinical point of view, the capacity of the graft to improve deficits in spontaneous behaviour probably is of critical importance in order to produce relief of symptoms in patients with Parkinson's disease.

Second, in animals with 6-OHDA or MPTP-induced Parkinsonism the fetal nigral grafts show long term survival and the functional effects are permanent unless the graft is removed. In contrast, the reduction of rotational asymmetry seen after implantation of adrenal medulla is transient and the chromaffin cells show atrophy unless NGF is supplied.

Third, the fetal DA neurons form appropriate synaptic contacts with the host striatum and restore a well-controlled DA neurotransmission. Adrenal medulla cells, on the other hand, possibly operate via a diffuse, non-controlled release of catecholamines (see, however, ref 68). This difference could be of critical importance for the usefulness of the grafts in patients with Parkinson's disease.

Grafting experiments in humans—adrenal medulla

Despite the encouraging animal data obtained with fetal DA neurons, the first clinical trials with transplantation in Parkinson's disease were performed using the patient's own adrenal medulla to avoid the ethical and immunological problems linked to the use of human fetal tissue. Between 1982 and 1985 four patients with severe Parkinson's disease were sub-

jected to adrenal medulla autotransplantation using a stereotaxic procedure^{69 70} (fig 1). Two of them were grafted in the caudate nucleus and two in the putamen. No adverse effects of the transplantation were observed. There was a minor improvement of motor function lasting for about 2 months but at 6 months no positive effects remained. The long-term follow up of these patients has not indicated any influence of the adrenal medulla autotransplantation on the natural course of the disease.

The results from these trials, which did not give anything of long-lasting therapeutic value to the patients, supported the idea that the symptoms of Parkinson's disease can be improved, seemingly with very little risk for the patient, through implantation of catecholamine-producing cells in the basal ganglia. It seemed clear, however, that the transplantation procedure had to be improved before any further clinical trials could be carried out.

In April 1987 Madrazo and co-workers reported the first successful adrenal medulla autotransplantations in two young patients with Parkinson's disease.⁷¹ Instead of a stereotaxic approach Madrazo and coworkers used open microsurgical techniques and through the cerebral cortex implanted pieces of adrenal medullary tissue into a premade cavity in the head of the caudate nucleus (fig 1).

After Madrazo's report was published the number of Parkinson's disease patients subjected to intracerebral grafting increased rapidly (fig 2). Whereas only single cases were operated in 1982 to 1985, about 15 patients received implants in 1986 and about 100 patients in 1987 and 1988. In the majority of patients the adrenal medullary tissue has been implanted into a cavity in the caudate nucleus according to Madrazo *et al.*⁷¹ but in a few cases successful attempts to use stereotaxic surgical techniques have been reported.⁷² Madrazo and co-workers have summarised⁷³ their findings from the first 22 patients subjected to adrenal medullary autotransplantation. Four of those patients have died due to cerebral venous thrombosis, bronchial aspiration, acute necrotic pancreatitis, and massive pulmonary thromboembolism, respectively.⁷³ Eighteen have been followed clinically for more than one year postoperatively. According to the scoring system of Madrazo *et al.*⁷³ nine of the patients had a poor and nine patients a moderate performance before surgery but after transplantation 13 patients had a good, four a moderate and one patient a poor performance. From these data it can be concluded that at least about 80% of Madrazo's patients have improved after surgery. The improvement has been described as a persistent reduction (at least up to 27 months) of tremor, rigidity and akinesia both during on and off periods. The daily doses of levodopa have been reduced by about 57% and two patients are

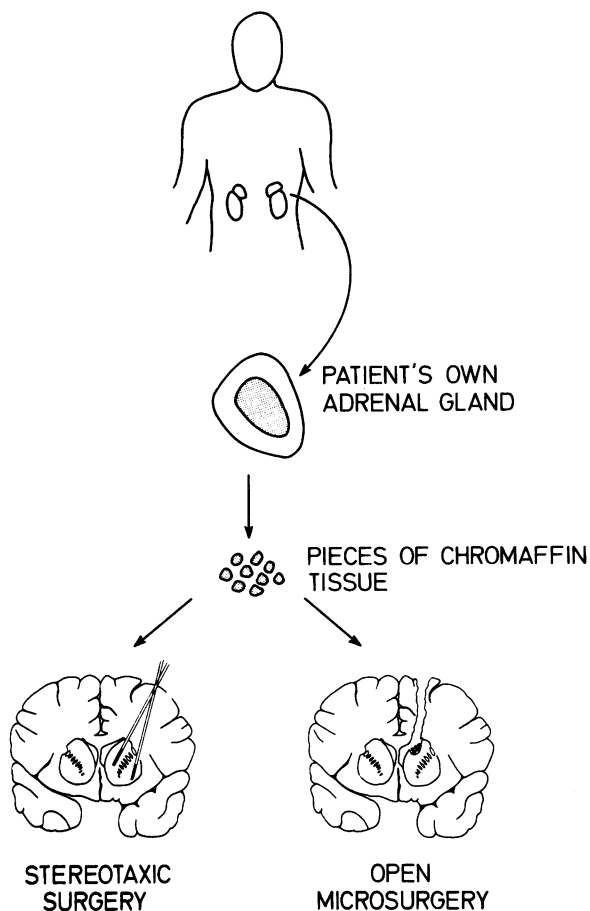


Fig 1 Schematic representation of the two alternative procedures used for grafting of adrenal medulla cells into the striatum of patients with Parkinson's disease. Pieces of chromaffin tissue are either implanted into the caudate nucleus or putamen using stereotaxic surgical techniques or with open microsurgery into a premade cavity in the head of the caudate nucleus.

without levodopa medication. Madrazo and co-workers⁷³ have reported that in the 18 surviving cases the complications of the procedure were transient and disappeared within one to two weeks. They included psychiatric disturbances with hallucinations, stupor, delusions, perseveration, respiratory problems, and pulmonary thromboembolism. The one exception was a patient who sustained damage to the fornix during operation and who had memory impairment.

About 250 operations using adrenal medullary autotransplantation have been carried out in several countries but mainly in Mexico and the United States. The scientific reports from these trials are very few and

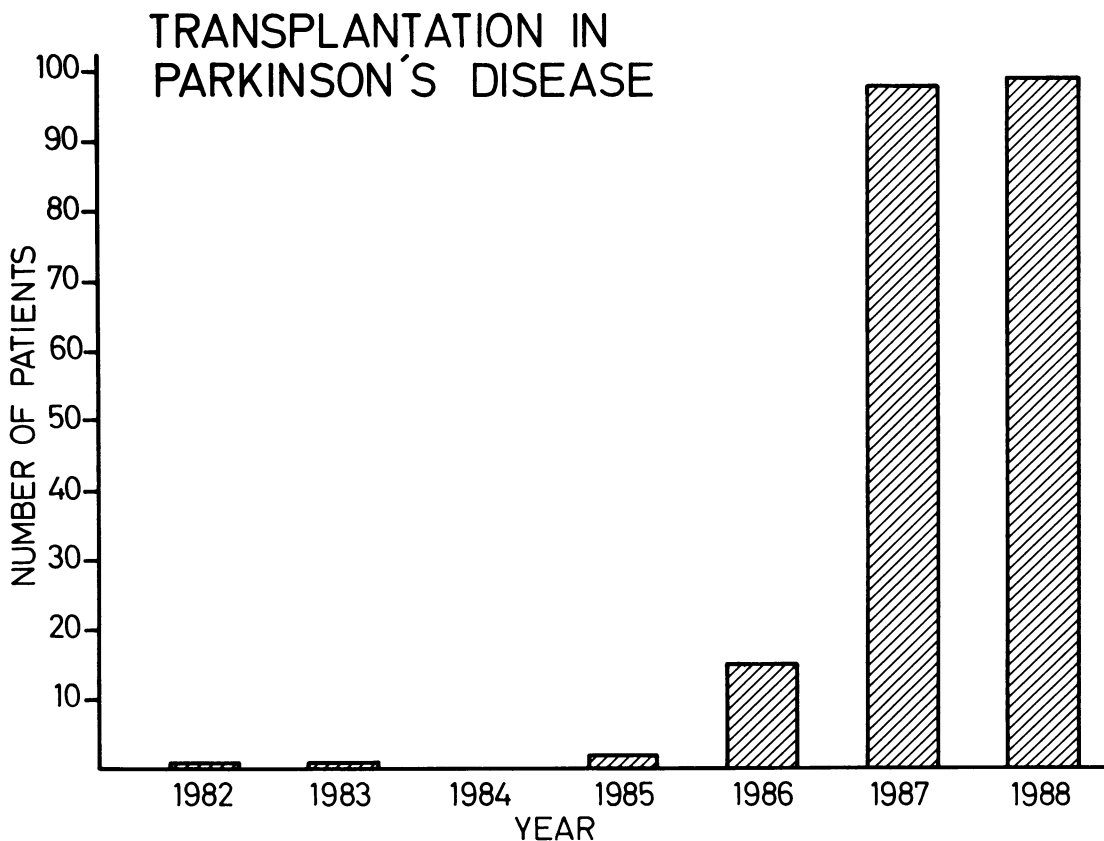


Fig 2 Estimated numbers of Parkinson's disease patients subjected to intracerebral grafting of catecholamine-producing cells into the striatum. In most cases the implanted tissue was the patient's own adrenal medulla but about 50 patients have received grafts of human fetal mesencephalic tissue.

the available data mainly derives from the experience in the United States. From the preliminary results of about 90 operations recently reported by different groups in the United States (Third United Parkinson Foundation Workshop on Brain Implants, Chicago, November 1988), it can be stated that only about 40% of the patients show some improvement. It has not been possible to reproduce the dramatic improvements reported by Madrazo and co-workers.^{71,73} In a detailed analysis^{74,75} of severely disabled Parkinson's disease patients followed for six months after adrenal medulla autotransplantation, performed according to the technique of Madrazo *et al.*,⁷¹ a modest reduction of the duration and severity of "off" periods was observed. The mean percentage of "on" time during the day increased from 48% before transplantation to 75% at six months after surgery. Even if the patients were more independent during "off" periods, they remained prominently affected by their disease. The

dosages of antiparkinsonian drugs could not be decreased after transplantation.

When evaluating the relative value of this procedure it is of course also important to note that the mortality rate, as well as the serious morbidity rate, as estimated from the results presented by different groups in the United States, is as high as 5 to 10%. A majority of patients show postoperative psychiatric and respiratory disturbances.⁷⁴ Furthermore, it is a serious drawback with this procedure that the mechanisms of improvement are largely unknown. As will be discussed below, it seems unlikely that the reduction of Parkinsonian symptoms is due to catecholamine release from the implanted cells as originally intended. It is furthermore not clear whether survival of the graft is necessary. In conclusion, open microsurgical autografting of adrenal medulla must be regarded as an experimental approach and not an established treatment for Parkinson's disease.

Grafting experiments in humans—fetal substantia nigra

On the basis of our own experience with adrenal medulla autotransplantation in patients^{69,70} as well as the more solid data obtained with grafting of fetal cells in experimental Parkinsonism (see above), a programme was initiated in Sweden in 1985 in order to apply the neural grafting technique in patients with Parkinson's disease. In 1985 the ethical question was debated in the Swedish Society of Medicine and this led to the adoption in 1986 of provisional ethical guidelines for the use of human fetal material for transplantation purposes. In summary, according to these guidelines women undergoing abortion must give their consent before tissue material, taken from the dead fetuses, can be used for transplantation. The transplantation cannot in any way influence how, when and why the abortion is carried out. No connection between a particular donor and a recipient is allowed.

In a series of experiments directly related to the clinical application of the neural grafting technique in Parkinson's disease, human fetal DA neurons were implanted into the DA-denervated striatum of immunosuppressed rats.⁷⁶⁻⁸⁰ The optimal donor age was found to be eight to 10 gestational weeks (fig 3A and B), and ventral mesencephalic tissue from fetuses of these ages (but not from older ones) survived transplantation and had functional effects. The DA cells reinnervated the entire rat striatum forming a terminal pattern resembling the intrinsic one (fig 4A-E) and with synaptic contacts with host striatal neurons (fig 5A, B). The human DA-rich grafts spontaneously released DA and reversed deficits in both drug-induced and spontaneous rotational

behaviour (fig 6). The ventral mesencephalon was intact and could be used for implantation into a Parkinsonian patient in about 50% of the abortions, which were carried out by suction (the abortion technique used for the fetal ages optimal for DA neuron survival). Repeated cultures showed that the entire procedure could be carried out without bacterial contamination.

In November and December 1987 two 50 year old women with Parkinson's disease since 1973 received intrastriatal grafts of human fetal DA neurons (for more details see ref 81). Using stereotaxic surgical techniques (fig 7) ventral mesencephalic tissue from four 8-10 week-old fetuses were implanted as a cell suspension on the side contralateral to that with the most severe symptoms of Parkinson's disease. The suspension was implanted at two sites in the putamen (tissue from one fetus in each) and at one site in the caudate nucleus (tissue from two fetuses). The patients were put on immunosuppressive treatment at the time of transplantation using standard regimens of cyclosporin, azathioprine and prednisolone. The same doses of antiparkinsonian drugs were given during the six months preceding the transplantation and during the six months follow-up period.

The results from up to six months postoperatively can be summarised as follows.⁸¹ First, there has been no marked changes in the percent time of the day the patients spend in an "on" phase; Second, small improvements have been noted in clinical tests of the motor performance of both patients during off periods. In patient 1 this slight bilateral improvement began at about three months after transplantation and was more marked contralateral to the implanted side. The motor performance of patient 2 was more

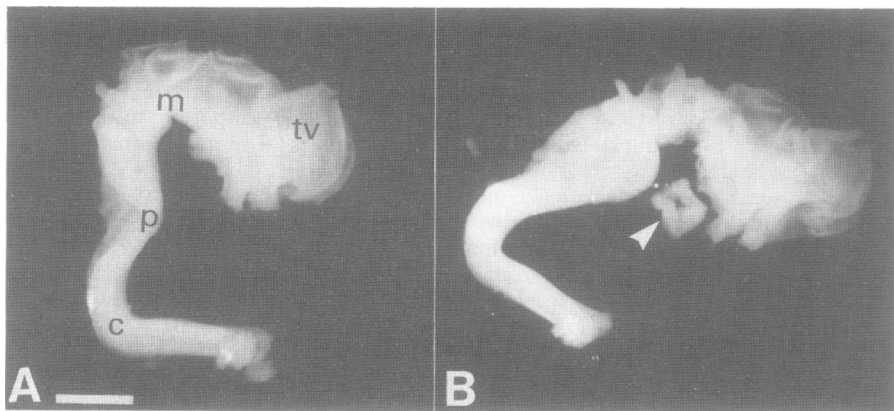


Fig 3 (A) The dissected central nervous system from a human fetus obtained at a suction curettage abortion (9 weeks gestational age). In (B) the ventral mesencephalon (arrow-head) has been dissected out. tv = telencephalic vesicle, m = mesencephalic flexure, p = pontine flexure, c = cervical flexure. Scale bar = 2 mm. From ref 78.

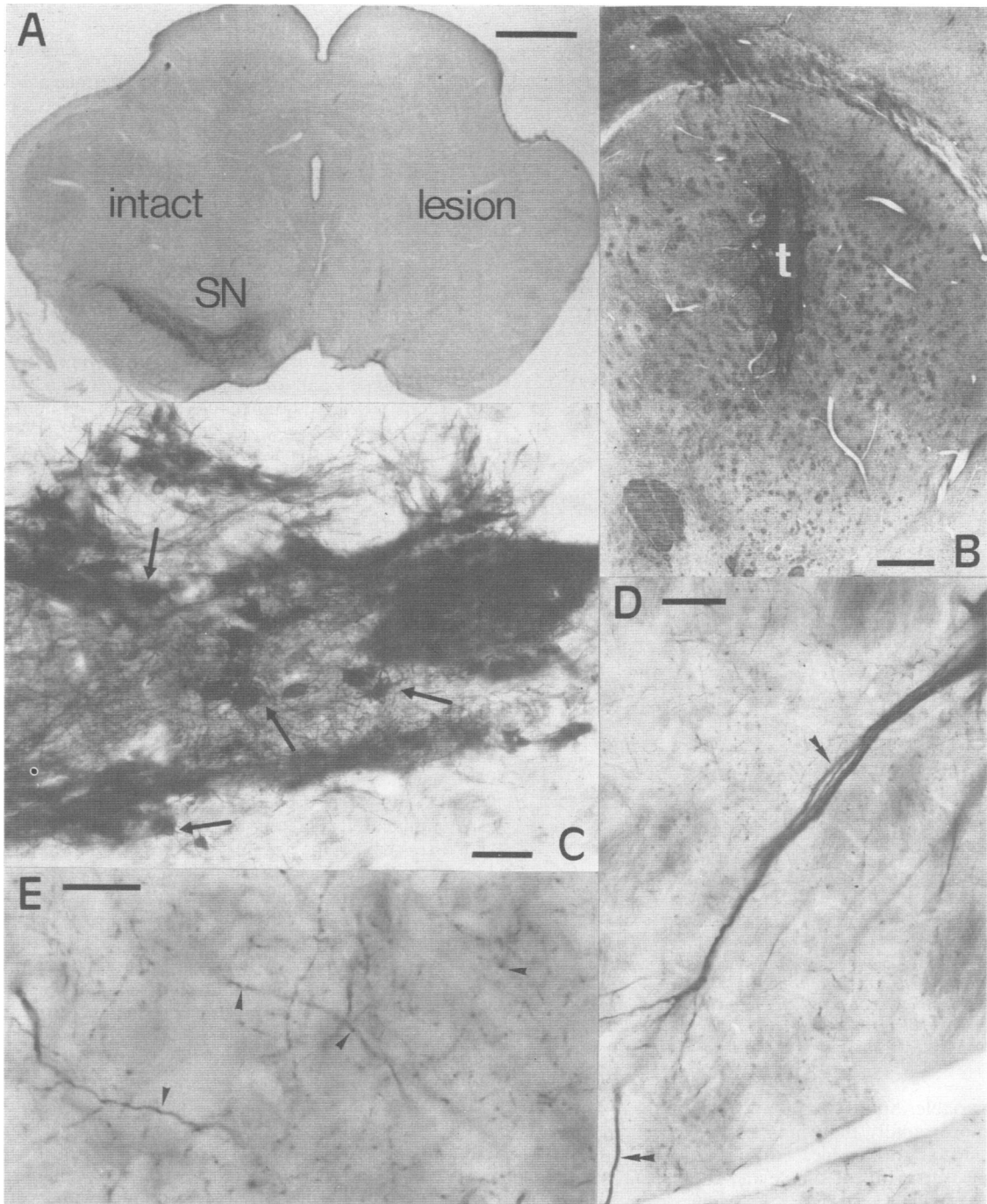


Fig 4 (A–E) Light microscopic pictures of sections, stained for tyrosine hydroxylase (TH) immunocytochemistry to demonstrate presumed DA neurons, from an immunosuppressed rat receiving human fetal mesencephalic grafts (20 weeks survival after implantation). (A) Overview of a section through the mesencephalon showing the effects of the unilateral 6-OHDA lesion. On the intact side, numerous DA neurons are seen in the substantia nigra (SN) and ventral tegmental area. On the side of the lesion virtually no TH-immunoreactivity is visible. (B) Overview of a section through the grafted caudate-putamen, illustrating the size and position of a transplant (t; from a human fetus, 10–12 weeks gestational age) 20 weeks post-grafting. (C) Higher magnification of a group of presumed DA neurons (arrows) situated within the human mesencephalic graft illustrated in (B). The graft is also seen to have a very dense plexus of TH-positive fibres. Note that the photograph is oriented so that dorsal is to the right and medial is upward. (D) Higher magnification of a bundle of coarse processes, probably dendrites (double arrow heads), which extend into the host striatum from the graft. (E) High magnification of graft-derived fine-calibre TH-positive fibres (arrowheads) in the host caudate-putamen. Scale bars; (A) 1 mm; (B) 0.5 mm; (C) 75 µm; (D) 50 µm; (E) 15 µm. From ref 78.

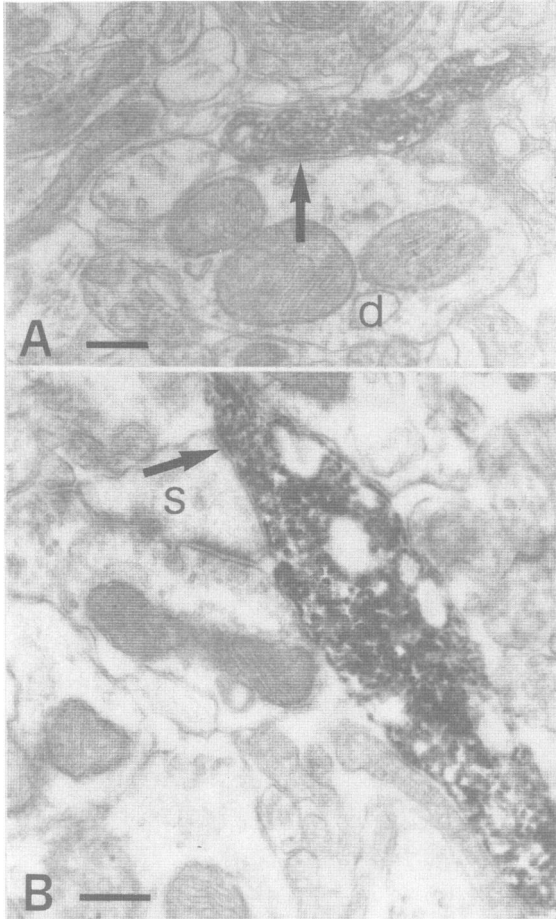


Fig 5 (A, B) Photomicrographs showing synaptic specialisations between grafted human DA neurons and host striatal neurons at 20 weeks post-grafting. In (A) a TH-positive bouton forms a symmetrical synapse (arrow) onto a dendritic shaft (d) and in (B) a large TH-immunoreactive fibre forms a symmetrical synaptic contact (arrow) with a dendritic spine(s). Scale bars: (A) and (B) 0.25 μ m. From ref 78.

variable, but at six months she performed the tests more rapidly than preoperatively, with no obvious side to side difference. However, in neurophysiological measurements of simple and complex arm and hand movements patient 2 showed a significant improvement on the side contralateral to transplantation but not on the ipsilateral side. Third, the duration of the response to a single levodopa dose did not change significantly in either patient; fourth, the motor readiness potential, which has been reported to be diminished in patients with Parkinson's disease,⁸² increased gradually during off in both patients; fifth,

positron emission tomography (PET) performed at 5–6 months postoperatively, did not show any increase of 6-L-(18F)-fluorodopa uptake in the grafted striatum.

The lack of a major clinical improvement in these patients can, in view of the PET data, most likely be ascribed to insufficient number of surviving grafted DA neurons. While this could be due to more general problems discussed below (for example, scaling up, immunological rejection, adverse effects on grafted cells by the continuous antiparkinsonian medication or the disease process itself), it also seems possible that technical factors such as the diameter of the implantation instrument and the time interval between the abortion and implantation could have been of importance.

Clinical trials with neural transplantation in Parkinson's disease are also going on in Mexico,⁸³ England,⁸⁴ USA, Spain, People's Republic of China, and Cuba. About 50 patients with Parkinson's disease have so far received intrastriatal implants of human fetal DA neurons. Both stereotaxic injection of a DA rich cell suspension into striatum (see for example, ref 84; fig 7) and implantation of pieces of ventral mesencephalic tissue into a cavity in the caudate nucleus (see ref 83; fig 7) are being used. No detailed scientific reports from these studies have yet been published but the preliminary descriptions of the results indicate in some cases much more marked improvements than was observed in the two Swedish patients.

The reasons for the discrepancies between the results obtained by different groups are unknown. One major difficulty, at present, is that the procedures used for assessment of the patients' symptoms as well as the time the patients have been followed preoperatively differ markedly between various studies. This means that the reported data are difficult to compare. There have been few attempts to demonstrate survival and ingrowth of grafted DA neurons, for example, with PET. Thus, it is not known if the reported improvements are to any extent dependent on restoration of DA transmission in the striatum as intended, or due to some other mechanism (see below). It seems unlikely that the early improvements, occurring within days to weeks, reported by Madrazo *et al*⁸³ and Hitchcock *et al*,⁸⁴ are due to a graft-derived reinnervation of the striatum. There is in human-to-rat grafting experiments a close correlation between the beginning of fibre ingrowth and synapse formation with host striatal neurons at about 2–3 months, and the onset of functional effects.^{77–78} (fig 6).

Possible mechanisms of action of catecholamine-producing cell implants

A major problem when analysing the data reported from the human trials is that graft survival and graft-

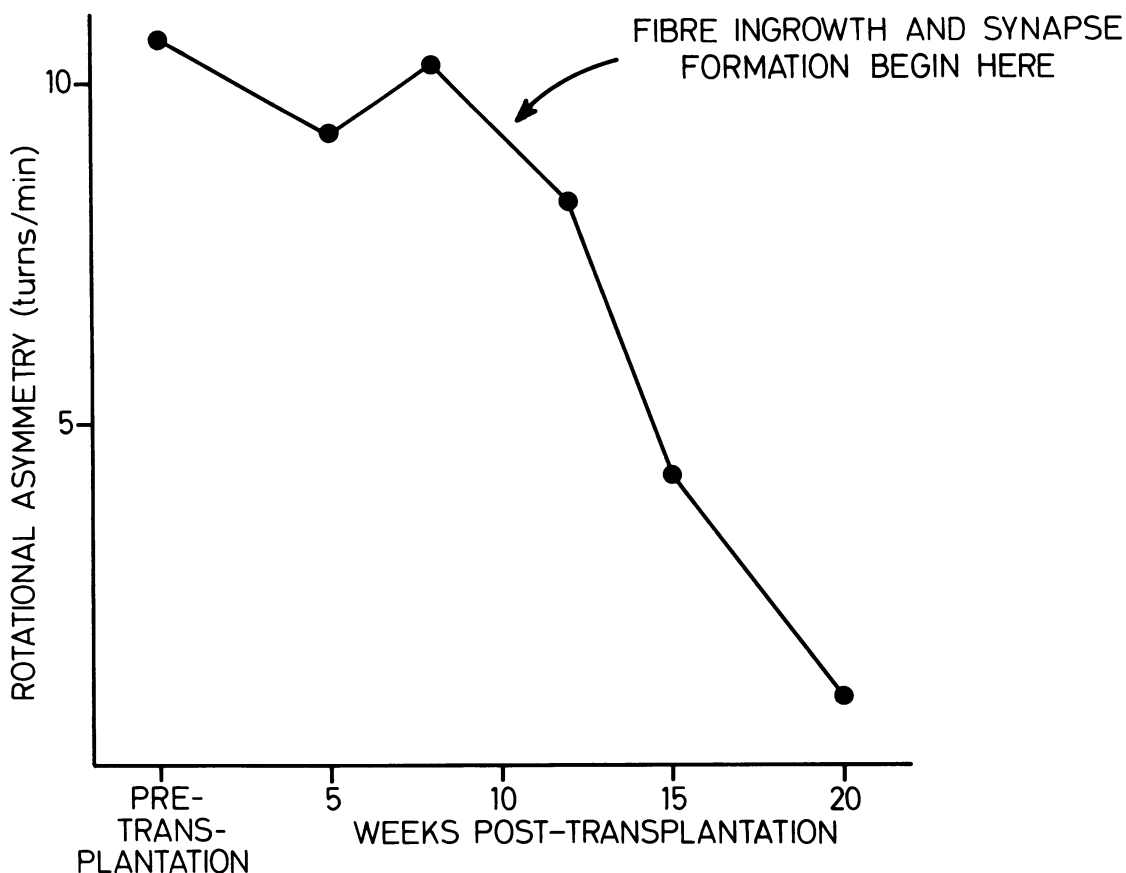


Fig 6 Relationship between the time course of development of functional effects and that of DA fibre ingrowth and synapse formation after implantation of ventral mesencephalic tissue from a human fetus into the DA-depleted striatum of immunosuppressed rats. The amphetamine-induced net rotation asymmetry scores (turns contralateral to the lesion side subtracted from turns ipsilateral to the lesion side) are shown both before and at various time points after transplantation for a group of unilaterally 6-OHDA denervated rats that received DA-rich implants from a 9 week old human fetus.⁷⁶ Morphological data from ref 78.

induced reinnervation have not yet been convincingly demonstrated in any of the operated Parkinson's disease patients. In the few cases that have been subjected to autopsy (all implanted with adrenal medullary tissue in the caudate nucleus according to Madrazo *et al*⁷¹) either no surviving graft^{85,86} or a very limited number of presumed adrenal medulla cells⁸⁷ (chromogranin A-positive but tyrosine-hydroxylase negative) have been demonstrated. Studies performed with PET in a few patients with grafts of adrenal medulla⁸⁸ or fetal nigra⁸¹ have not detected any signs of increased catecholaminergic function in the striatum after transplantation (as assessed by the uptake of 18-F-fluorodopa and 11-C-nomifensine). It should be pointed out, though, that most of the cases analysed

have shown very little clinical benefit from the operation (which agrees with a poor graft survival) and it can therefore not be excluded that in the more successful cases there is also a higher yield of surviving catecholamine-producing cells. Unequivocal demonstration of graft survival and reinnervation of the host striatum remains, however, one of the major scientific challenges in future clinical trials with transplantation in Parkinson's disease.

What are the possible mechanisms of action of catecholamine-rich cell implants underlying the observed improvements in patients with Parkinson's disease? If other explanations for the clinical changes can be excluded, such as placebo responses, or manipulations with antiparkinsonian drug treatment, there

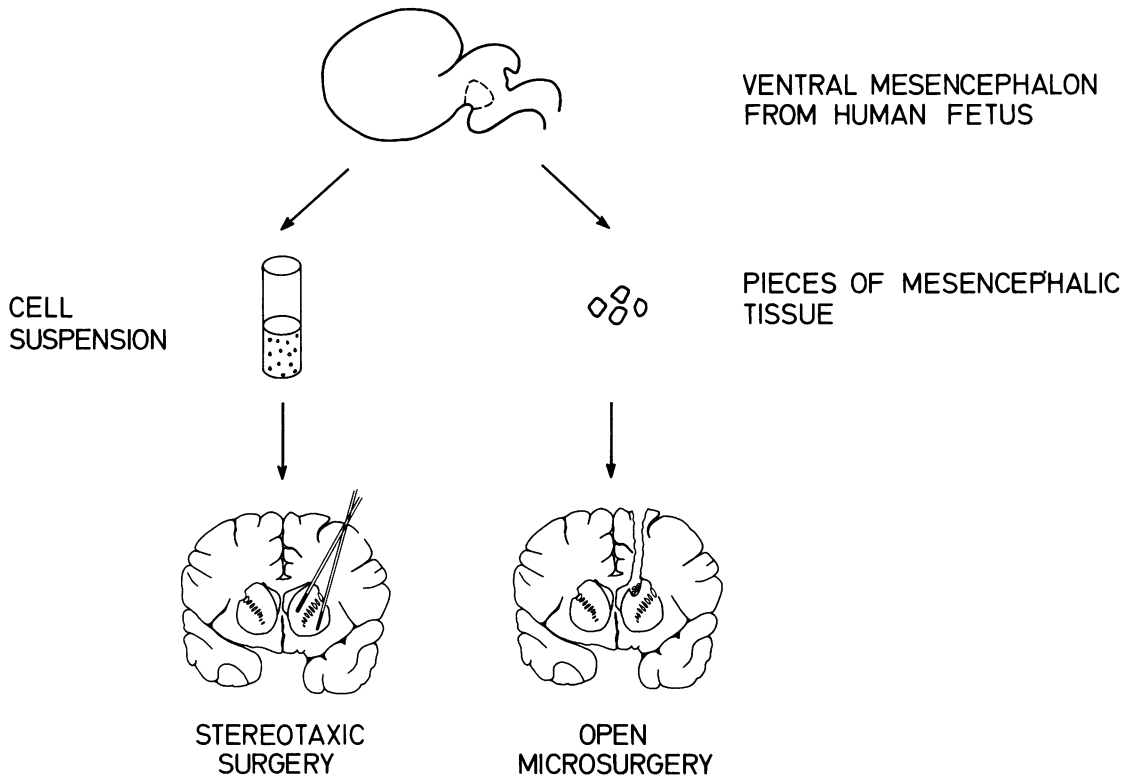


Fig 7 Schematic representation of the two alternative procedures used for grafting of human fetal DA neurons into the striatum of patients with Parkinson's disease. The ventral mesencephalon is either prepared as a cell suspension which is implanted stereotaxically into the caudate nucleus and/or putamen, or divided in pieces which are put into a premade cavity in the head of the caudate nucleus.

are three major possibilities (fig 8A–C): *First*, the grafts could work as biological minipumps and release DA (or other catecholamines) diffusely into the striatum or into the cerebrospinal fluid (fig 8A). This has been reported to occur when rat adrenal medulla cells are implanted into the rat striatum,⁶⁴ in which case a leakage of transmitter into the surrounding host tissue can be demonstrated. Such a diffuse catecholamine release has been proposed to account for the functional effects observed after adrenal medulla autotransplantation, at least in rats. This is supported by the finding that chronic infusion of DA into the denervated striatum using a mini-pump is able to reduce apomorphine-induced rotational asymmetry to the same degree as intrastriatal adrenal medullary grafts.⁸⁹ Indeed, the first human trials were carried out with the intention of utilising this mechanism also in the Parkinsonian brain but so far there is little evidence that the grafted chromaffin tissue works in this way in patients except perhaps during a transient period.⁷⁰ Analyses of catecholamines and their metabolites in ventricular cerebrospinal fluid follow-

ing adrenal medulla grafting according to Madrazo *et al*⁷¹ have not demonstrated any significant increases even in patients who have exhibited clinical improvement.^{90–92}

Second, the grafted cells may grow into the host striatum and restore synaptic neurotransmission (fig 8B). This mechanism of action seems more likely with implants of fetal neural tissue than after adrenal medulla autotransplantation (except possibly in a future clinical setting when NGF is administered to the graft). There are, however, no PET data available to support the idea that the improvements reported after grafting of fetal mesencephalon can be attributed to a graft-derived increase of dopaminergic nerve terminal density in the striatum. Furthermore, as pointed out above, the time course of the improvements reported from the patients operated in Mexico⁸³ and England⁸⁴ differs markedly from that of ingrowth and synapse formation from human fetal DA neurons^{77,78} (at least when xenografted into the rat, fig 6).

A third possibility is that the graft, at least for a time,

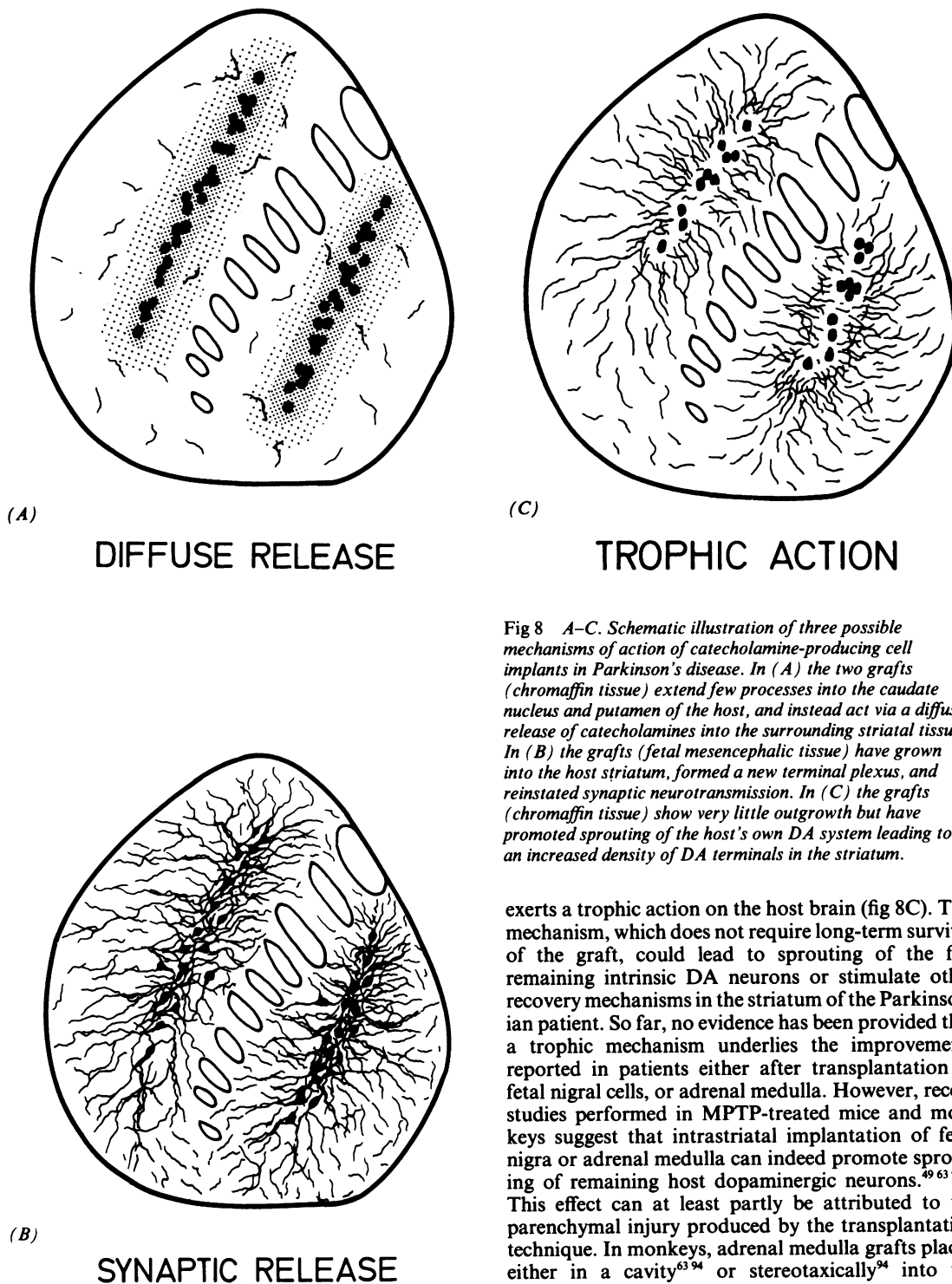


Fig 8 A-C. Schematic illustration of three possible mechanisms of action of catecholamine-producing cell implants in Parkinson's disease. In (A) the two grafts (chromaffin tissue) extend few processes into the caudate nucleus and putamen of the host, and instead act via a diffuse release of catecholamines into the surrounding striatal tissue. In (B) the grafts (fetal mesencephalic tissue) have grown into the host striatum, formed a new terminal plexus, and reinstated synaptic neurotransmission. In (C) the grafts (chromaffin tissue) show very little outgrowth but have promoted sprouting of the host's own DA system leading to an increased density of DA terminals in the striatum.

exerts a trophic action on the host brain (fig 8C). This mechanism, which does not require long-term survival of the graft, could lead to sprouting of the few remaining intrinsic DA neurons or stimulate other recovery mechanisms in the striatum of the Parkinsonian patient. So far, no evidence has been provided that a trophic mechanism underlies the improvements reported in patients either after transplantation of fetal nigral cells, or adrenal medulla. However, recent studies performed in MPTP-treated mice and monkeys suggest that intrastriatal implantation of fetal nigra or adrenal medulla can indeed promote sprouting of remaining host dopaminergic neurons.^{49 63 93 94} This effect can at least partly be attributed to the parenchymal injury produced by the transplantation technique. In monkeys, adrenal medulla grafts placed either in a cavity^{63 94} or stereotactically⁹⁴ into the

caudate nucleus induced a presumed sprouting response of DA fibres to an extent that corresponded to surgical procedures without any tissue implants. The survival of adrenal chromaffin cells in the grafts was minimal.^{63,94} According to Bankiewicz *et al*⁴⁹ fetal nigral tissue is more effective and gives rise to extensive sprouting of presumed dopaminergic neurons in the host brain.

Some critical issues for successful clinical application of the neural grafting technique

Scaling up. Most experimental studies with neural transplantation have been carried out in rodents and only a few in monkeys. In the rat striatum between 100 and 200 surviving DA cells are necessary in order to effect a greater than 50% reduction of amphetamine-induced rotational behaviour in hemiparkinsonian rats.^{95,96} This number of cells constitutes only about 1–2% of the neurons forming the intrinsic mesostriatal DA system in the rat.⁹⁷ Given the rat data, what is the critical number of surviving grafted DA neurons needed in the human brain to obtain a therapeutic effect? Optimally, 20 000–25 000 DA cells from both sides of the mesencephalon of each human fetus (8 to 10 gestational weeks) survive grafting to the striatum of immunosuppressed rats.⁷⁷ Since it can be estimated⁹⁸ that the human putamen and caudate nucleus are normally innervated by about 60 000 DA neurons each, grafting of ventral mesencephalic tissue from one fetus into one of these structures might be able to restore 30 to 40% of the normal number of cells. The symptoms of Parkinson's disease do not appear until more than 70% of the DA neurons have degenerated^{99,100}; until this stage is reached, DA transmission is believed to be maintained through hyperactivity of remaining DA neurons and post-synaptic receptor supersensitivity.^{101,102} It therefore seems realistic to postulate that tissue from one or more human fetuses, implanted unilaterally into the putamen, caudate nucleus or both, would be sufficient to effect some symptomatic improvement for a patient with Parkinson's disease.

It should be remembered, though, that the volume of the putamen is 200 times larger in man than in the rat brain. To what extent this structure will be reinnervated is not only dependent on the number of surviving grafted DA neurons but also on the number and location of implantations and the growth capacity of each DA neuron. Grafted rat DA neurons extend their fibre network about 1.5–2 mm from the graft border,¹⁹ whereas human DA neurons implanted into the rat striatum have exhibited a growth distance of at least 3 mm,⁷⁷ that is until the growing fibres reach the border of the striatum. The human DA neurons thus have a higher growth capacity than rat DA neurons but the maximum extension of their axonal processes

is not known. This is, of course, of great importance in order to decide the number of implantation sites. Before we performed our first grafting experiments in patients, we estimated that each human DA neuron could grow up to 4.1 to 5.4 mm. Theoretically, about 40 to 80% of the volume of the putamen would then be reached by the growing DA axons implanted along two injection tracts. The lack of any major effect could in part be due to an overestimation of the growth capacity of human DA neurons and may indicate that a larger number of implantation sites are necessary.

Host environment. A common feature of the experimental models of human neurodegenerative disorders used in grafting experiments is that the exposure to the causative agent (for example, 6-OHDA, MPTP) occurs during a short period of time and has ceased at the time of cell implantation. In idiopathic Parkinson's disease the aetiology is unknown and it may therefore be hypothesised that the disease process itself could interfere with the long-term survival and outgrowth of DA neurons implanted into the patient's brain. This would most likely not occur if the degeneration is due to an intrinsic defect in their own DA system, such as a premature or accelerated aging process^{103,104} or a lack of scavenger molecules that can remove endogeneously produced free radicals.^{105–107} If, on the other hand, Parkinsonian patients lose DA neurons because of a disease process within the striatum but extrinsic to these neurons, for example to a lack of trophic substances, this process could also elicit a degeneration of the grafted neurons. Similarly, if the DA neurons have degenerated due to an exogenous insult, of which the causative agent were still present, this process could also cause a gradual death of DA neuron in the graft or could inhibit growth. This would be the case if the patient continues to be exposed to some environmental toxin (see refs 108–110). However, if the degeneration resulting from this exposure is a slow process, one would expect that the patient should still benefit from DA neuron grafting, at least for a limited time period.

In severely affected Parkinson's disease patients, drug therapy must be maintained until beneficial effects appear, which would be expected at about three months postoperatively. However, the grafted neurons may be vulnerable to this treatment, particularly during the period of nerve fibre formation. It has been suggested that levodopa treatment may accelerate the degeneration of remaining intrinsic nigrostriatal DA neurons in Parkinsonian patients (see ref 111). This might occur, for example, through an autoxidation of levodopa which has been reported to produce many cytotoxic substances such as free radicals and quinones.¹¹² One cannot rule out the possibility that levodopa treatment interferes with the survival and growth of the grafted DA neurons; this

possibility should be tested in animal experiments.

Immunosuppression. Although the brain is often considered to be immunologically privileged (see refs 113 and 114) it is now well established that immunological rejection processes occur also in the CNS.^{36 115 116} Fetal neural tissue has been shown to be immunogenic^{116 117} and can express major histocompatibility complex (MHC) antigens (the strong transplantation antigens) after the proper inductive stimuli. In fact, in vitro expression of MHC antigens has been demonstrated after intracerebral grafting.¹¹⁶ There is evidence of host immunisation by fetal neural grafts of human origin in a xenograft situation.⁷⁷ Immune reactions as well as fulminant rejections have been observed in animal recipients of human fetal neural tissue.⁷⁷

However, implantation of allografts of fetal substantia nigra into the brains of MPTP-lesioned non-human primates has in most cases been performed without immunosuppression. The available reports have indicated good graft function up to at least seven months postoperatively.^{47 48} Only one case of re-grafting has been described in monkeys in which a prompt loss of graft function was observed on the previously implanted side.¹¹⁸ It is not known if immunosuppression is necessary in clinical trials with grafting of human fetal neuronal tissue. However, it seems at present appropriate to use such treatment in order to optimise the condition for graft survival and to increase the chance for successful re-grafting. If immunosuppression is necessary a major problem is that, at present, there are no non-invasive approaches to monitor rejection of grafted fetal tissue in the brain. If it precedes any functional effects, rejection would probably pass unnoticed. If positive functional effects have developed, rejection would lead to a partial or complete disappearance of such graft-induced improvements. In either situation it would be desirable to detect the first signs of a rejection process in the brain in order to be able to prevent the rejection of the graft.

Concluding remarks

Much experimental evidence, derived from several animal models, suggests that cell transplantation into the adult CNS has a great potential to become valuable in the treatment of a variety of human neurological disorders. Although grafting of fetal neurons seems to be the most promising approach at present, other sources of cells, such as adrenal medulla or cultured cell lines, may provide alternative strategies. In the future, the use of genetically engineered cells for transplantation purposes also seems to be a realistic possibility.¹¹⁹ However, it must be concluded that at present cell transplantation into the human brain is at an early, experimental stage. The mechanisms underlying the reported improvements after transplantation in patients with Parkinson's

disease must be clarified in detail. Above all, it remains to be demonstrated that grafted cells can survive permanently in the human brain affected by various disease states. Obviously, for further progress in this field, it will be vitally important that basic animal studies are carried out in parallel to well-designed human trials.

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