

FROM NEURONS TO PILLS

A SYMPOSIUM ON A REVOLUTIONARY METHOD IN
NEUROBIOLOGY AND THE ROLE OF CREATIVE
RESEARCH ENVIRONMENTS



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Cell Transplantation for Parkinson's Disease in Lund.

Some personal recollections

Anders Björklund and Olle Lindvall

Introduction

When it was introduced in the 1960-ies, the Falck-Hillarp histofluorescence method opened entirely new possibilities for the study of monoaminergic systems. It was unique not only in its ability to visualize neurons on the basis of their neurotransmitter content, but it was also the first neuroanatomical method that allowed selective visualization of neurons and their projections in the microscope based on their transmitter phenotype. During the first few years after its introduction, the method was used in anatomical and pharmacological studies of catecholamine- and serotonin-containing neurons in a broad range of animal species. Soon, however, it was realized that the selectivity of the histofluorescence technique, and its ability to visualize the catecholamine neurons in their entirety,

Anders Björklund and Olle Lindvall are professors at the Faculty of Medicine at the University of Lund. They have both participated significantly in the development of basic and clinical neurobiological sciences at the University of Lund. In 2000 they jointly received the prestigious Söderberg prize from the hands of princess Lilian, as seen in the picture to the right.

Both obtained their PhD degrees at Bengt Falck's Department of Histology. Anders Björklund remained at the same department, whereas Olle Lindvall went to clinical neurology. However, they maintained close ties, and here describe how their successful program for restorative neurology emerged.



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including cell bodies as well as axons, offered unique possibilities for studies of neuronal growth and regeneration, not only during development, but also in the adult nervous system. In his PhD thesis work, Lars Olson was the first to take advantage of this favorable feature of the method in studies of regeneration of sympathetic axons in the peripheral nervous system, and he was also the first to use this method in experiments with transplants in the anterior chamber of the eye. During the 1970-ies, together with his PhD student Åke Seiger, he developed this ground-breaking approach further, using the intra-ocular transplantation technique as a tool for exploring the growth properties of noradrenergic and dopaminergic neurons, “cultured”, so to speak, on the richly vascularized iris in the eye of adult rats.

In Lund, it was an American guest scientist, Robert Katzman, who triggered our interest in neuroregeneration. In 1969-70 he spent a sabbatical in Bengt Falck's lab at the department of Histology. In brain sections from rats subjected to an electrolytic lesion of the nigro-striatal pathway and treated with the Falck-Hillarp method, he made the serendipitous observation that nigral dopamine neurons exhibited a surprisingly abundant and extensive sprouting after axotomy. He asked one of us (AB) to join in the further analysis of this phenomenon. This interesting, and indeed highly unexpected finding, which was published in *Brain Research* in 1971, was the start of a series of studies on axonal regeneration of axotomized monoamine neurons in the brain and spinal cord.

At the time, the prevailing view of neuronal regeneration was that it occurred in some cold-blooded vertebrates, but that in mammals efficient regeneration was confined to the peripheral nervous system. In studies going back to the classic experiments of Santiago Ramon y Cajal, regrowth of axons after damage in the adult mammalian brain or spinal cord was seen as abortive, at best. Sustained regeneration and regrowth of axons over longer distances, as we could report in our studies of regenerating monoaminergic neurons in the adult rat CNS, was thus highly unexpected. Equally unexpected was the remarkable growth of transplanted fetal noradrenergic, dopaminergic, serotonergic and cholinergic neurons as well as neuroblasts. Their capacity to provide new, functional innervations of previously denervated regions in the adult CNS, such as hippocampus, striatum and spinal cord, is a striking demonstration of the ability of the damaged brain to integrate new cellular elements into functional circuitry. These early studies received much attention and opened up for new ideas of how cellular implants, such as cells derived from stem cell sources, may be used to help the brain to repair itself.

Parkinson's Disease

Of the wide range of experimental approaches pursued during these pioneering years there is one that has had particular impact, i.e., the use of cellular transplants for dopamine neuron replacement in Parkinson's disease. The development of cell transplantation in this disease is an interesting example of how basic science research over a relatively short time span can be translated towards clinical application. One reason why this happened in Sweden is the fact that the interactions between clinical and experiments investigators is, by tradition, very close at Swedish universities, and not least in Lund. The collaboration between the two of us started early, already during our medical studies, and ten years later, at the time when cell transplantation became "hot", we had published extensively together, mostly in studies on the anatomical organization of the dopamine and noradrenaline systems in the brain. It therefore came very naturally to us to join forces in this endeavor, and our close and friendly interactions with our colleagues at the Karolinska Institute in Stockholm turned out to be an asset.

In this article we have summarized the events that led to the development of this approach and its pioneering use in clinical trials in Parkinson's disease patients. We have chosen to do this in the form of our personal recollections; the development of the pre-clinical studies, written by AB, and the initiation and development of the clinical trials, written by OL. The personal perspective we have adopted here makes our story decidedly "Lundo-centric". Important contributions have been made by other researchers at various stages of this development. For a more complete coverage of the development of the cell transplantation field we recommend the reader to turn to the more scholarly reviews published elsewhere.

How the pre-clinical work evolved

Anders Björklund

Transplantation in the nervous system is a classic approach in cold-blooded vertebrates, such as urodeles, amphibians and fish, and it goes back to the early decades of the last century. Roger Sperry's experiments in the amphibian visual system showing restoration of vision after transplantation, or re-implantation, of eyes after optic nerve transection, performed in the 1940-ies, is a particularly interesting example. In mammals, the early studies by Elisabeth Dunn and Wilfried LeGros Clark had shown that tissue taken from the developing brain can survive transplantation to the brain of early postnatal rats, and studies of neural tissue transplanted into the anterior chamber of the eye had given similar results.

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These early reports had provided proof-of-principle that tissue pieces dissected from the developing nervous system can survive transplantation, continue deve-

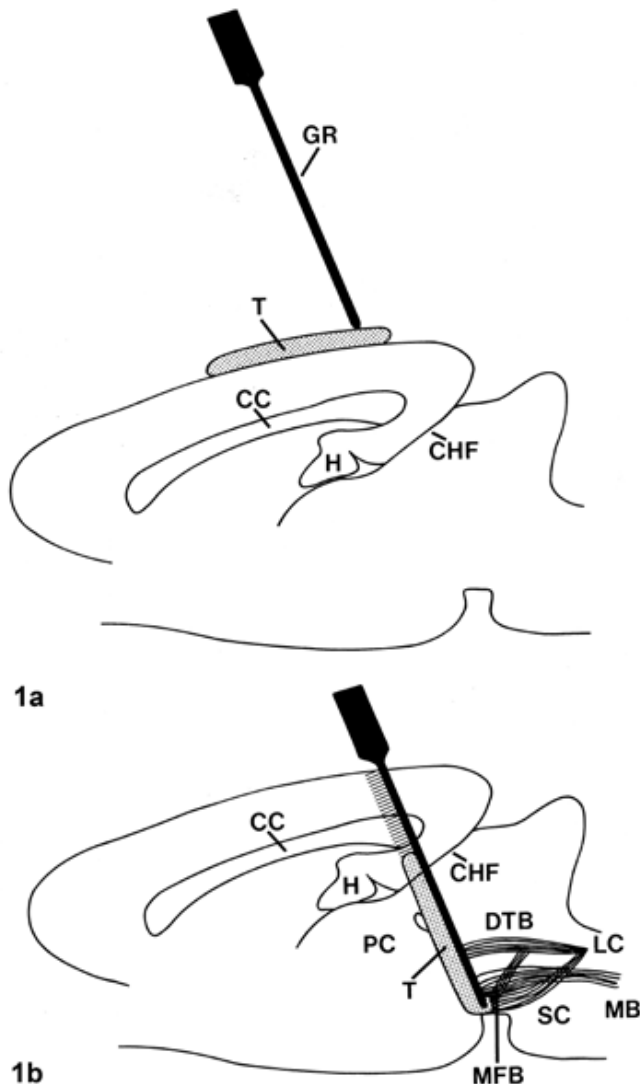


Figure 1 (left). The technique used in the early experiments to transplant iris tissue to the midbrain in adult rats. The iris sheet is placed flat on the dorsal surface (a) and gently pushed into the brain parenchyma using a flat glass rod (b). The rod transects the dorsal noradrenergic bundle (DTB) and the medial forebrain bundle (MFB), and leaves the iris (T) in direct contact with the transected axons derived from the noradrenergic neurons in the locus coeruleus (LC).

Figure 2 (right). The transplanted iris could be removed and unfolded, allowing the unfolded tissue sheet to be spread as a whole-mount on a microscopic slide. In this way the extent of regeneration of the lesioned locus coeruleus neurons could be visualized in its entirety. (From: Svendgaard, Björklund and Stenevi, *Advances In Anatomy Embryology and Cell Biology*, Springer Verlag, vol. 51, no 4: 1-76, 1975).

lopment in their new location and retain at least some aspects of their internal organization and cellular composition. However, due to the limitations of the microscopic techniques used, these studies did not provide any information on the ability of the transplanted neurons to integrate into host brain circuitry or establish connections with the surrounding host tissue.

How it started

In 1972 a paper published by our close colleagues at the Karolinska Institute in Stockholm, Lars Olson and Åke Seiger, published in *Zeitschrift für Zellforschung* (vol. 135: pp 175-194), caught my attention. This paper reported results from a series of experiments where small pieces of tissue, dissected from the brainstem of rat fetuses, were transplanted to the anterior chamber of the eye in adult rats. The pieces were dissected to include midbrain or pontine nuclei containing developing noradrenergic, dopaminergic or serotonergic neurons. This study took advantage not only of possibility to monitor the survival and growth of the transplanted tissue piece non-invasively through the cornea, but more importantly of the fact that the Falck-Hillarp histofluorescence method made it possible to visualize selectively the monoamine-containing neurons – their cell bodies and outgrowing axons – in their new location on top of the host iris. In a flash, I realized that this approach should be possible to use also in studies of transplants in the brain. Provided, of course, that transplants of developing monoamine neurons could be made to survive inside the brain.

My ally in the lab at that time was my close friend and collaborator Ulf Stenevi. He was just finishing his doctoral thesis work, which he carried out in parallel with his medical studies. This had involved a study of the capacity of lesioned noradrenergic axons, originating in the locus coeruleus, to re-innervate the smooth muscle in pieces of iris transplanted to the midbrain in adult rats. The technique used in these experiments involved a thin, flat glass rod which was used to gently push the thin iris tissue vertically into place through an opening in the skull (Figure 1). The slit lesion made by the rod was positioned such that it transected the axons emanating from the locus coeruleus at the level of the midbrain, running in the so-called dorsal noradrenergic bundle (marked DTB in Fig. 1b). At the same time, the thin iris tissue was left in direct contact with the transected axons. The results obtained in these experiments, published in *Brain Research* in 1971 (31:1-20), showed that the lesioned locus coeruleus axons were able to regenerate extensively into the iris muscle, forming a terminal network reminiscent of the original sympathetic noradrenergic innervation, thus providing a striking demonstration of a previously unrecognized regenerative capacity of noradrenergic neurons in the brain. If the transplants were analyzed within the

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first 4 weeks after implantation, the grafted iris could be removed and unfolded, and the unfolded sheet of iris muscle could be spread out as a whole-mount on a microscopic slide and reacted with formaldehyde gas according to the Falck-Hillarp method. The images of the regenerated adrenergic axonal network we obtained that way were absolutely amazing. An example of this is seen in the whole-mount picture illustrated in Figure 2.

The first attempts to transplant neurons to the brain

Our first attempts to transplant monoamine neurons to the brain made use of this rod-implantation approach. We started with pieces of sympathetic ganglia and pieces of brain tissue dissected from fetal ventral midbrain, transplanted to the midbrain in adult rats. The grafted pieces of fetal ventral midbrain did not survive. Of the 48 sympathetic ganglia transplants, 11 showed some survival. In the surviving cases we observed that the transplants had received a vascular supply either from pial vessels located on the ventral brain surface, or from the choroidal fissure, i.e. the obliterated fissure separating hippocampus from the underlying diencephalon, suggesting that survival was critically depending on access to blood supply. In other experiments Ulf had used suction lesions of the cerebral cortex in adult rats and he knew that the richly vascularized surface overlying the midbrain tectum could be readily exposed through a suction cavity made in the retrosplenial cortex. Ulf now suggested that we used that approach to generate a “culturing chamber” for the transplanted tissue pieces, as illustrated in Figure 3,

analogous to the anterior eye chamber where the pieces were “cultured” on the surface of the richly vascularized iris.

This approach worked beautifully, and over the following two years we made a series of experiments with transplants of small dissected pieces of fetal brainstem tissue contain-

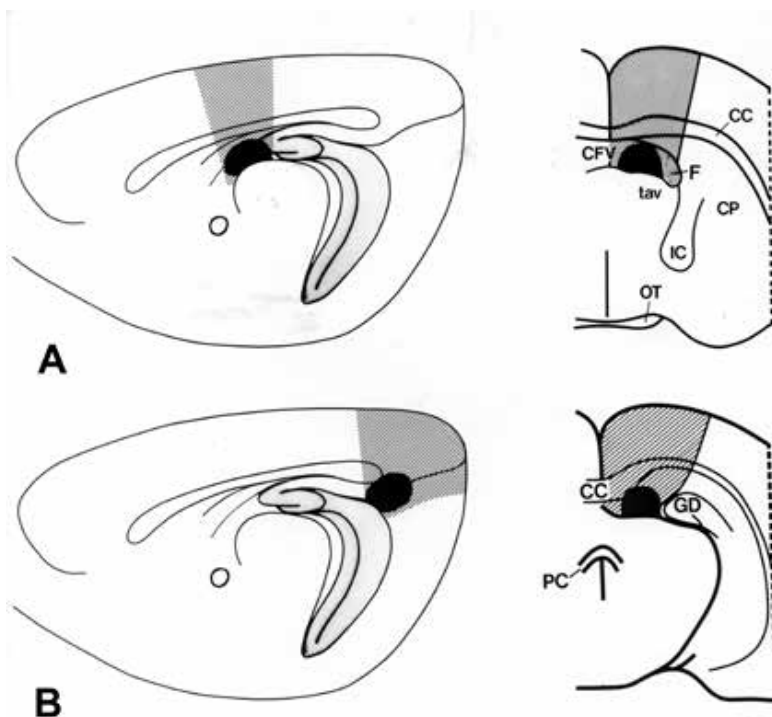
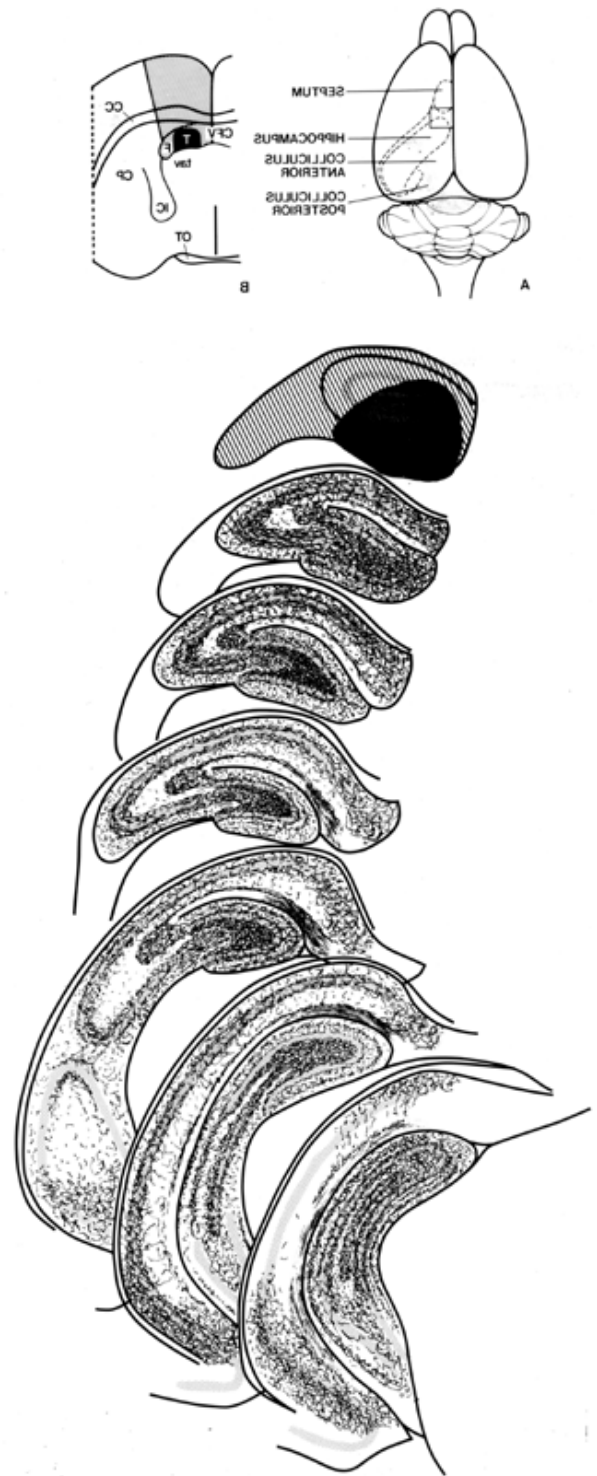


Figure 3. In the initial studies of intracerebral transplants we “cultured” the tissue pieces on the vascular surface present at the bottom of suction cavities made in the parietal cortex (A) or the retrosplenial cortex (B).

*Figure 4. Transplants of fetal monoamine or cholinergic neurons were amazingly efficient in re-innervating the previously denervated hippocampus. This camera lucida drawing shows the extent and pattern of transplant-derived cholinergic axons re-innervating the hippocampus (acetylcholine esterase staining). The graft, derived from the fetal septal-diagonal band area, is shown in black, and the hippocampus is represented in six equally spaced frontal planes from rostral (top) to caudal (bottom). (Adapted from Björklund and Stenevi, *Cell and Tissue Research*, 185:289-302, 1977).*



ing developing noradrenergic, dopaminergic or serotonergic neurons, as well as transplants of fetal cholinergic neurons obtained from the septal/diagonal band area from the developing basal forebrain, published between 1976 and 1979. The results published in *Nature* in 1976 (262:787-790), in *Cell and Tissue Research* in 1977 (185:289-302), and in *Brain Research* in 1979 (170:409-426) were particularly striking, showing a remarkable capacity of the grafted noradrenergic and cholinergic neurons to re-innervate the denervated hippocampus in a neuron-specific manner and form electrophysiologically normal functional contacts with the neurons in the host. As illustrated in Figures 4 and 5, the innervation patterns of the intrinsic noradrenergic and cholinergic innervations (removed by lesions prior to transplantation) were very accurately reproduced by the re-innervating axons derived from the grafted locus coeruleus and septal neurons, respectively. These exciting findings opened up a fascinating field of study on the functionality of the newly formed graft-host connections, and their impact of hippocampus-related behaviors, that we were fortunate to carry out in collaboration with some of the best scientists in the field, Stephen Dunnett, Rusty Gage and Menahem Segal, in particular. This line of

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Figure 5. Innervation of the hippocampus derived from a graft of adult superior cervical ganglion, taken from the same animal and placed in a retro-splenic cavity, as described in the text. (From: Björklund et al., Nature 262: 787-790, 1976).

research, however, is outside the scope of the history told here.

Transplantation in the rat Parkinson's disease model

The study of fetal ventral midbrain grafts showed that the transplanted dopamine neurons survived well in the retrosplenic suction cavity, and that they grew axons into the surrounding host brain. This location, however, is remote from their normal targets, the striatum in particular. Our first attempts to transplant fetal ventral midbrain dopamine neurons to the striatum were performed in 1975, but without success. The approach we used in these first attempts was to prepare a suction cavity in the parietal cortex, through the corpus callosum, so as to expose the dorsal surface of the caudate-putamen. In this location the cavity is lacking the vascular surface present on the surface of the tectum present at the bottom of the retro-splenic cavity, thus failing to provide the necessary vascular support. We reported these observations in our 1976 Brain Research paper (114: 1-20) and then left it aside in favor of the studies on transplants innervating the hippocampus that worked so well. Three year later we took it up again. After some trials we found that we could make the fetal ventral midbrain grafts to survive by performing the surgery in two steps: In a first step the cavi-

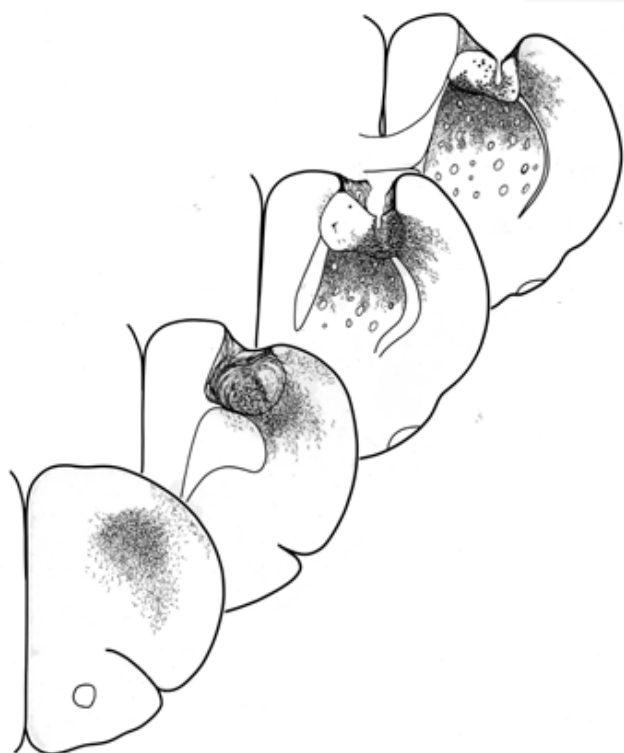


Figure 6. When placed in a cavity made on top of the striatum, grafts of fetal ventral dopamine neurons were efficient in re-innervating the previously denervated striatum in 6-hydroxydopamine-lesioned rats. The functional recovery seen in the grafted rats was well correlated with the extent of fiber outgrowth in the underlying striatum. (Adapted from Fray et al., Science 219:416-419, 1983).

ty was prepared, filled with gel-foam and closed. Three to four weeks later the cavity was re-exposed and the fetal ventral midbrain piece was placed over the vascular bed that had developed in response to the lesion. Now the grafts survived well and grew in size, and we saw that the dopamine neurons were capable to provide the underlying striatum (previously denervated by a unilateral 6-hydroxydopamine, 6-OHDA, lesion) with a new dense innervation that covered most of the dorsal caudate-putamen in the host. Figures 6 and 7 give examples of such two-stage ventral midbrain transplants, and the extensive axonal outgrowth obtained from the grafted dopamine neurons.

We published these initial findings in *Brain Research* in 1979 (177: 555-560). We submitted this paper first to *Science*, but while it was

under review a similar study, to our surprise, appeared in *Science* (Perlow et al., 204: 643-646). As a result, being too similar, the editors kindly declined our paper, and we rushed to have it published in *Brain Research* instead. This competing study, which was performed by a young US investigator, M. J. Perlow, in collaboration with the Karolinska team, was based on the same idea but the approach was different. In the Perlow et al. study fetal ventral midbrain tissue was implanted into the lateral ventricle, in contact with the medial surface of the denervated caudate-putamen. These transplants turned out to be quite small, and in contrast to the effective re-innervation seen in our transplanted animals,



Figure 7. Detail of a ventral midbrain transplant, processed according to the Falck-Hillarp method, showing the survival of dopamine neurons and the associated graft-derived dopamine innervation of the underlying striatum (CP).

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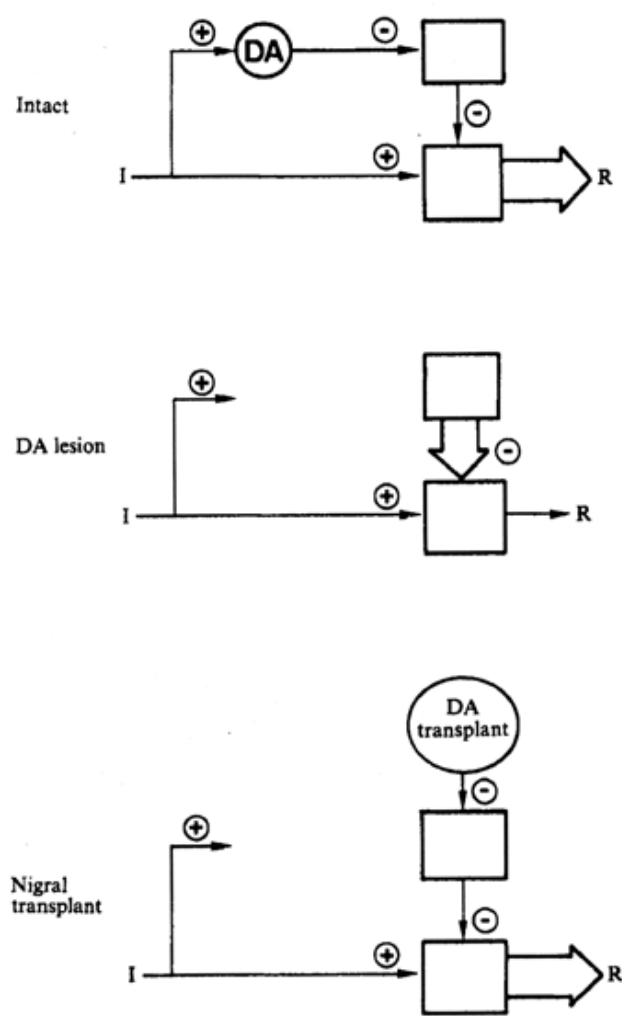


Figure 8. Mode of action of dopamine neuron transplants, as discussed further in the text. According to this model, published in our 1976 Nature paper, the transplanted dopamine neurons restore the ability of the animal to initiate movements (R) in response to sensory stimuli (I). This mode of action explains why dopamine neurons can be functionally efficient even when they are in the wrong place, i.e. in striatum rather than in their normal location in the substantia nigra.

the growth of axons from the intraventricular transplants into the striatum was quite limited. We found that the functional impact of our intra-cavity grafts, monitored by tests of amphetamine-induced turning, was directly correlated to the extent of striatal re-innervation, and in the most successful cases the motor asymmetry seen in the unilaterally lesioned rats was completely reversed. This led us to propose that the grafts of fetal dopamine neurons could be used to establish a new functional "nigro-striatal" connection able to compensate for the missing dopamine innervation in the toxin lesioned animals. The observations reported in the Perlow study suggested a different functional mechanism. The functional response induced by the intraventricular transplants, as monitored by apomorphine-induced turning, was seen to depend on the location of the graft rather than on the extent of fiber outgrowth, suggesting that the functional response was due to dopamine diffusing from the grafted cells into the adjacent denervated striatum. Apomorphine-induced turning is known to depend on the presence of supersensitive dopamine receptors present in the denervated striatum, and the reduced turning seen in animals with intraventricular transplants is thus most likely due to normalisation of receptor sensitivity by dopamine diffusing from the CSF into the striatal parenchyma. This interpretation of the observations made in Perlow et al. study was further corroborated in a subsequent study the NIMH/Karolinska team showing that a similar effect on apomorphine-induced turning could be obtained by intraventricular transplants of adrenal chromaffin cells, i.e. by cells lacking any axonal projections (Freed et al., Nature 292:351-352).

To develop our approach further we teamed up with Susan Iversen in Cambridge, UK, one of the leading researchers in the study of the function of dopamine neurons in the brain. I met Sue at the Ciba Foundation (later Novartis Foundation) in London. In a break at the meeting I asked her whether she would be interested in a collaboration to explore the functionality of intra-cerebral transplants, and she was immediately positive. She engaged a newly recruited PhD student, Stephen Dunnett, in this work. This was the start of a long and fruitful collaboration that developed into one of my most rewarding and stimulating partnerships. Starting in 1979, Steve came on several month-long visits to Lund, and once he had passed his PhD in 1980, he spent a year as a postdoc in my lab, studying not only the impact of nigral transplants in the rat Parkinson's disease model, but also the functional effects of septal cholinergic neuron transplants in models of hippocampus-related memory and learning impairments. In 1980 our work got a tremendous boost when Rusty Gage joined the lab as a postdoc. This was a terrific addition and added great momentum to the development of these projects, and the intense and productive years that followed stand out as a golden time in my scientific life.

As a follow-up to the 1979 paper we published in 1980-81 six papers in rapid succession. Collectively, these studies provided an extended analysis of the survival, growth and effects of fetal nigral transplants on both drug-induced and spontaneous motor behaviors, as seen in rats with either unilateral or bilateral 6-OHDA lesions of the nigro-striatal dopamine pathway. In our 1981 *Nature* paper (289:497-470) we proposed a mechanistic model of dopamine graft function that was based on a model of dopamine neuron function previously proposed by Edward Stricker and Michael Zigmond. In this model (Figure 8) dopamine acts in the striatum as a tonic regulatory system that sets the activity in the movement-initiating cortico-striatal circuitry, and reinstatement of dopaminergic neurotransmission by dopamine neuron transplants is viewed as a re-activation of an inhibited, but otherwise intact, neuronal machinery, thus reducing the threshold for behavioral response to movement-activating sensory stimuli. According to this model, tonic, unregulated release of the transmitter at the re-innervated synaptic sites would be sufficient to induce recovery of sensori-motor behavior, at least in the rat Parkinson's disease model. Subsequent studies of graft-induced activity and dopamine release have added further support for this mode of action, which helps to explain why dopamine neuron transplants placed in an ectopic location (i.e. in the striatum rather than in their normal location in the ventral midbrain) are capable of restoring at least some aspects of normal dopamine-dependent behaviors.

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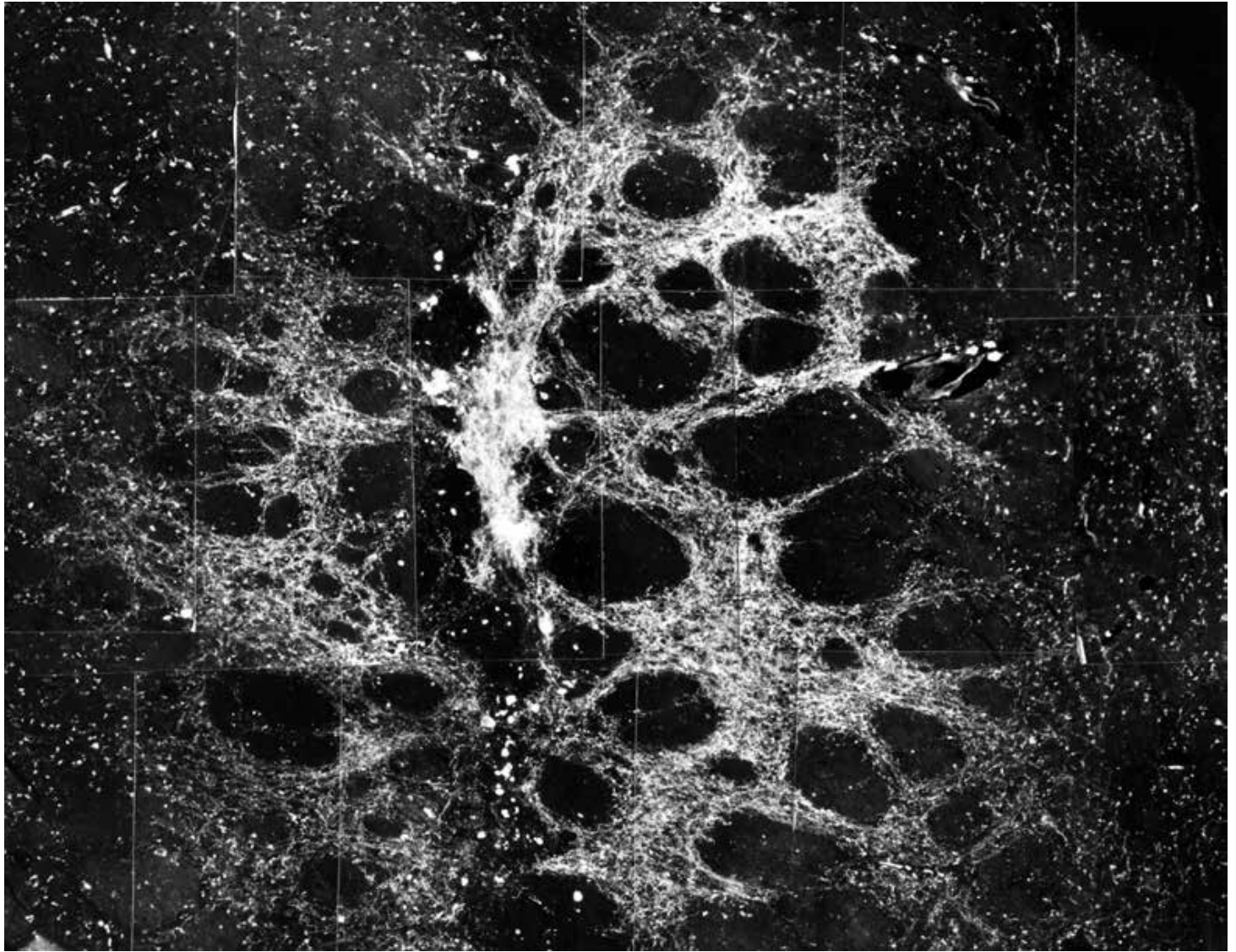


Figure 9. Intrastratial graft of nigral dopamine neurons, visualized with the Falck-Hillarp technique. From the cluster of surviving dopamine neurons (in the center of the picture) graft-derived axons have grown to innervate the surrounding striatal tissue. The black “holes” are the myelinated axon bundles of the internal capsule, running through the striatum. (From: Gage et al., Science 221, 966-969, 1983).

In 1981 we abandoned the cavity transplantation procedure in favor of a less invasive technique based on stereotaxic injections of dissociated cell suspensions directly into the brain parenchyma. Solid pieces of tissue was known to do well inside the cerebral ventricles, but survived poorly in brain parenchyma. We reasoned that the ability of the cells to survive in the absence of access to CSF would improve if the cells were implanted in dispersed form. The procedure we used – trypsinization and mechanical dissociation using a fire-polished Pasteur pipette - was taken from a paper by Banker and Cowan used in a study of fetal hippocampal neurons in dispersed cell culture. Typically, 3-5 μ l of cell suspensi-

on were injected at one or several sites in the striatal parenchyma, using a Hamilton high precision syringe. In this way, dopamine neuroblasts could be implanted into the denervated striatum with minimum trauma from the implantation needle. The survival of the grafted dopamine neuroblasts (about 5%) was sufficient to generate a dense re-innervation of the striatum surrounding the graft deposits (Figure 9). This allowed selective re-innervation of defined parts of both caudate putamen and nucleus accumbens, as well as more complete re-innervation of the striatum using multiple deposits.

We published this technique in a short paper in *Cell and Tissue Research* in 1980 (212: 39-45) and went on to characterize it in further detail in a series of papers published as a supplement to *Acta Physiologica Scandinavica* (Suppl. 522: 1-48, 1983). Here, we published an extensive study of the results obtained with the cell suspension technique in uni- or bi-laterally 6-OHDA lesioned rats. The report of these findings was divided into five parts and included both microscopic, biochemical and behavioral analyses of rats that had received cell suspension transplants in different locations in the dopamine-denervated striatum. The cell suspension technique was an important improvement in that it allowed implantation in selected brain regions and at multiple implantation sites, and made it possible to dose the amount of cells grafted in a more controlled way. This is nowadays the standard approach used for intracerebral transplantation in rodents and primates, and is also the method used in the clinical trials.

Exploration of human fetal tissue in preparation for clinical trials

In 1985 we got the permission from our local research ethics committee to use human fetal brain tissue, obtained from legally aborted fetuses, for transplantation in our rat Parkinson's disease model. At that time a bright young medical student, Patrik Brundin, had started as a PhD student in my lab. His thesis work was focused on a series of pre-clinical studies, partly using human fetal ventral midbrain tissue, in preparation for future clinical trials. In the studies on human fetal ventral midbrain tissue we teamed up with Olle Lindvall who had already established collaboration with Lars Olson and Åke Seiger in Stockholm in a project aimed at performing some of the first transplants of autologous adrenal chromaffin tissue in patients with Parkinson's disease (see below).

In three papers published in *Experimental Brain Research* (65:235-240, 1986; 70: 192-208, 1988; and 73: 115-126, 1988) we showed that human fetal ventral midbrain tissue from 7-9 week old fetuses survived well after transplantation to the striatum, provided that the animals were immunosuppressed by daily injec-

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tions of cyclosporine. The grafted neurons were efficient in re-innervating the previously denervated striatum, reverse amphetamine-induced rotation, restore dopamine release, and form normal synaptic contacts with the host striatal projection neurons. In these experiments we were also able to identify the appropriate landmarks to be used for dissection of the dopamine-rich ventral midbrain tissue pieces, and define the optimal range of donor ages to be used in the clinical protocol.

In parallel, and in collaboration with other labs, Patrik and I performed a series of studies on standard rat-to-rat ventral midbrain cell suspension transplants using microdialysis and in vivo voltammetry to monitor the extent of recovery of dopamine release, and with the use of ultrastructural and tract-tracing techniques we studied the integration of the grafted dopamine neurons into host striatal circuitry, the synapses made onto denervated striatal projection neurons in the host, and the extent of host afferent inputs onto the grafted dopamine neurons. In these studies we could show that the graft-derived dopamine innervation established normal synaptic contacts with the host striatal neurons (Freund et al., *J. Neuroscience* 5:603-616, 1985), and that the grafted dopamine neurons were able to restore striatal dopamine release to near normal levels (Forni et al., *Exp. Brain Research* 76:75-87, 1989). Further, the release rate was under regulation by autoreceptor-mediated feedback, as is the case in the intact system (Strecker et al., *Neuroscience* 22:169-178, 1987). In collaboration with Ron Mandel, who spent his postdoc in my lab in 1989-90, we studied the importance of graft placement and task complexity for transplant-induced recovery of simple and complex sensori-motor deficits in the rat Parkinson's disease model, published in *European Journal of Neuroscience* (2:888-894, 1990). But at that time, the clinical trials were already well under way, and the first positive findings were published in an article in *Science* (Lindvall et al, *Science* 247:574-577, 1990).

The clinical program

Olle Lindvall

At the time when the idea of cell replacement was introduced in the clinic, new therapeutic approaches for Parkinson's disease were highly warranted, especially in the so-called complication phase when the efficacy of the L-dopa treatment had started to decline. The new findings in animal models had obvious clinical implications, raising the possibility of the development of a transplantation therapy for Parkinson's disease patients. In 1979, I had been working as a clinical neurologist for 3 years, and was enthusiastic about the preclinical data but hesitant about the clinical translation for two main reasons: First, the practical

problem. Would it be possible to collect human fetal tissue from routine abortions, avoiding contamination, identify the ventral mesencephalon containing the dopaminergic neurons and then implant the tissue into the Parkinson's disease patient's brain without adverse effects? Second, and most important, the ethical problem. Would it be ethically and morally justified to collect and use tissue from dead, aborted human fetuses for intracerebral transplantation in Parkinson's disease patients in order to ameliorate severe motor symptoms? No guidelines for the use of such tissue were available. Although the use of human fetal tissue for transplantation would have to be approved by an ethical committee, it was necessary for me first to come to a personal standpoint. This was not easy and took time. After careful consideration, I came to the conclusion, which I have defended since then, that using human fetal tissue as well as human embryonic stem cells to relieve suffering in severely disabled patients is ethically and morally justified. I got a lot of support in this difficult process by my father who was a clergyman in the Swedish church.

The first transplantations in Lund

In 1983 and 1984, I listened to two inspiring lectures by Lars Olson about the pioneering transplantation studies that he had carried out together with Åke Seiger and Erik-Olof Backlund in two Parkinson's disease patients at the Karolinska Institute. This group had performed the first intracerebral human transplantations in the world in 1982 and 1983. The two patients had been implanted with tissue from their own adrenal medulla into the caudate nucleus. The clinical experiments were based on a paper in *Nature* (292:351-352, 1981) providing some evidence that catecholamines secreted from transplanted adrenal medulla tissue could reduce a rotational deficit in a rodent Parkinson's disease model. At a meeting in Stockholm, Lars and I started to talk about a possible collaborative clinical trial in which we would introduce two major

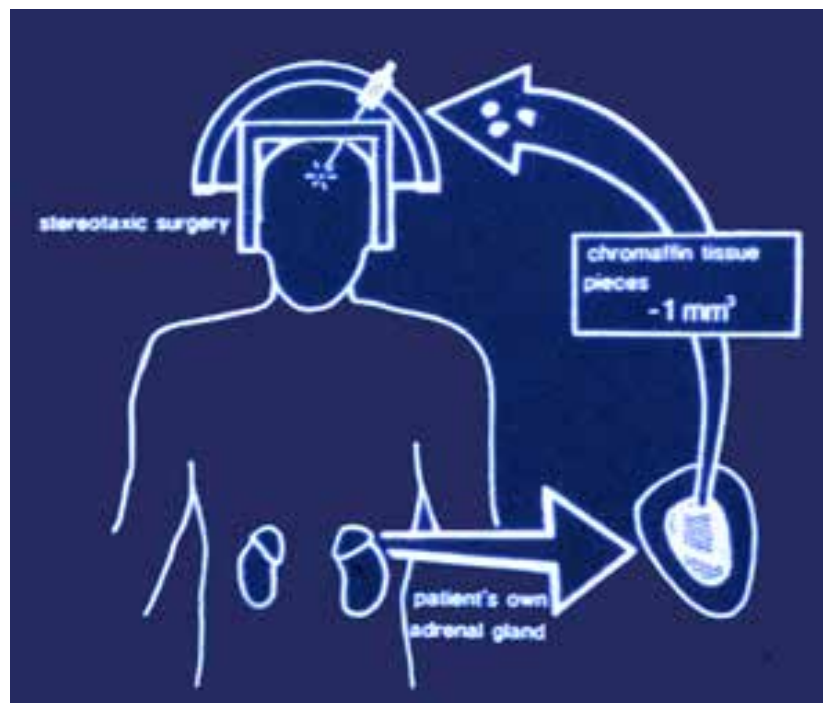


Figure 10. Cartoon showing principles for transplanting adrenal medullary tissue in patients with Parkinson's disease, as first done by Olson, Backlund and Seiger in Stockholm

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Figure 11. A patient selected for transplantation is performing a foot tapping test, which is video-recorded for quantification.

improvements in adrenal medulla auto-transplantation for Parkinson's disease: First, more careful and extensive neurological assessment in order to detect also minor and focal motor improvements. Second, implantation of the tissue into the putamen instead of the caudate with the objective to improve motor function. Two of my patients with severe Parkinson's disease were selected for transplantation.

The common Parkinson's disease rating scales were not particularly useful for evaluating the motor performance of our patients and demonstrate a possible graft-induced effect. We speculated that

by cell implantation unilaterally in the striatum, an improvement (if it indeed occurred), would be detectable primarily on the contralateral side, and perhaps only in the upper or lower limb. For the evaluation after transplantation of adrenal medulla and later also of fetal mesencephalic tissue, we therefore developed a battery of quantitative, timed motor tests, e.g., 20 pronations/supinations, fist clenches, finger dexterity and foot tapplings (Figure 11) together with standard neurological examinations. Patients scored whether they were mobile (in "on" phase) or had severe Parkinson's disease symptoms (in "off" phase) every 30 minutes. We also measured the duration of the effect of a single dose of L-dopa,

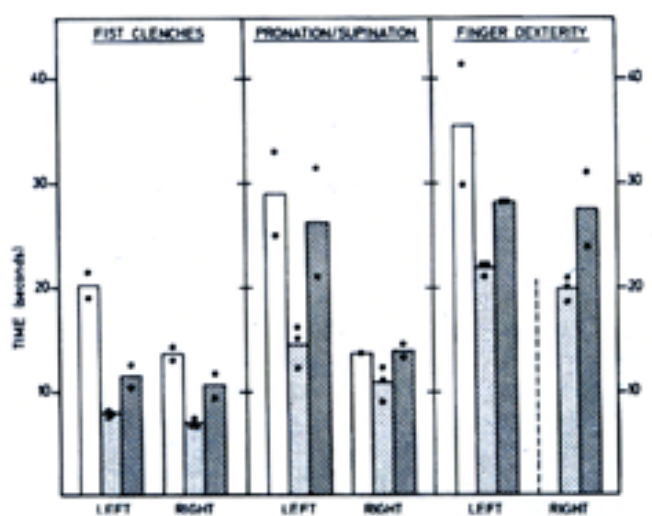
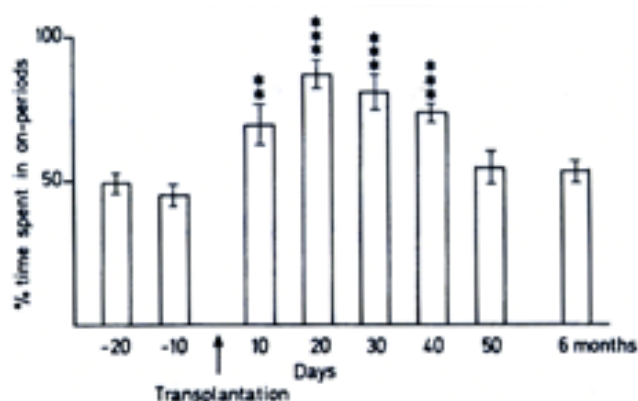


Figure 12. Autotransplantation of adrenal medulla in two patients with Parkinson's disease resulted in significant but transient improvements.

because we hypothesized that the graft would increase the brain's buffering capacity for L-dopa and thereby prolong its effect.

On April 19, 1985, the first cell transplantation was performed at the University Hospital in Lund. Two teams were working together: a surgical team headed by Anders Nobin (a close friend and long-term collaborator) removed one adrenal gland, which was dissected by Åke Seiger and Lars Olson. The neurosurgeon Erik-Olof Backlund then took over and implanted the tissue unilaterally into the putamen of the patient. The surgery room was crowded with more than 20 people who wanted to be present on this unique occasion, the third "brain transplantation" in the world. We had a syringe with haloperidol available in the surgery room in case the patient would react with an acute psychosis due to excessive catecholamine release from the adrenal medulla graft. Nothing happened, however, but later in the evening, the patient showed signs of pulmonary edema, which was treated successfully. After careful consideration, we decided to go ahead with the next patient on the subsequent day. This transplantation session went well.

Ups and downs with adrenal medulla grafts

My first presentation of the data from our patients with adrenal medulla grafts took place at a meeting at the New York Academy of Sciences in 1986. The main findings, later published in *Annals of Neurology* (22(4):457-68, 1987), were a transient improvement of motor function (Figure 12), primarily on the side of the body contralateral to the graft. The reaction of the audience to my talk was overtly negative, and during the discussion I felt that people literally threw rotten tomatoes at me. The discussion was printed so we can still read, e.g., the following statements. C. Sotelo: "Having been a nerve biologist for 25 years, I would rather do experiments in animals than in humans"; M. J. Perlow: "You ascribe the beneficial result to catecholamine release. I do not find any evidence in your paper that there was any catecholamine release, and I wonder how you could come to that conclusion"; S. Fahn: "It always amazes me how careful basic laboratory scientists are to have controls in animal experiments, but once they start working on patients, the controls disappear out of the window and we're left with bad treatments for years". After this heavy criticism which really made me very worried, Patrik Brundin (who then was a 24-year old PhD student) gave some memorable advice to all of us, thereby rounding off the discussion: "I suggest that the clinicians should go to a laboratory and look down a microscope and the neurobiologist should go to a neurological clinic and look at a patient."

After my talk, I was quite depressed. In my view, our study had given some valuable information. First, that it was possible to implant cells in the human puta-

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men without adverse effects. Second, that our assessment protocol and test battery could detect small changes in motor function in the Parkinson's disease patients. But was it possible to move forward with clinical transplantation when the recommendations from the meeting were to perform many years of preclinical experiments, including studies in monkeys? Anders and I shared hotel room during the meeting and we were discussing a lot during the night following my talk. During that night we decided to go ahead with our clinical transplantation program using human fetal mesencephalic tissue, despite the resistance in the scientific and clinical community,

Everything was then altered by an article published in the New England Journal of Medicine on April 2, 1987 (316: 831-834). In this article, the Mexican neurosurgeon Ignacio Madrazo and his colleagues reported that implantation of adrenal medulla tissue into a cavity in the head of the caudate nucleus had led to dramatic improvements in two severely disabled Parkinson's disease patients. A world-famous movement disorder specialist who visited Madrazo said when he returned to New York "I have seen history". Personally, I was very surprised and also disappointed because I could not understand the mechanisms underlying the reported major improvement, especially in the light of our own marginal and transient effects with adrenal medulla grafts. However, the scientific and clinical communities were excited and most of those colleagues who had been extremely critical when I presented our data in New York the year before now just wanted to go ahead quickly with adrenal medulla transplantation. At the transplantation meeting in Rochester later in 1987, Madrazo and his collaborator were kings. Unfortunately, criticism was regarded as expression of chauvinism. At a video-session, one of Madrazo's patients showed his tremor and it was definitely not a typical Parkinsonian tremor, which I pointed out. My comment was regarded as very negative and one famous neuroscientist stated that my criticism was only due to the fact that the responsible scientist was Mexican and Spanish-speaking. This scientist did not speak to me for several years.

The first transplantations of dopamine-rich fetal mesencephalic tissue

The planning of the first transplantations with human fetal mesencephalic tissue was carried out with support from the Swedish Medical Research Council by researchers from Karolinska Institute, including Lars Olson, Åke Seiger, Ingrid Strömberg and Erik-Olof Backlund (later in Bergen), and from Lund Anders Björklund, Patrik Brundin, Stig Rehnström, Håkan Widner, Birger Åstedt and myself. (Figure 13) Several productive meetings were held in Stockholm and Lund. During this time, Erik-Olof Backlund decided to leave the program due to



Figure 13. Dinner with the group planning tissue transplantations for Parkinson's disease, March 9th, 1987. Around the table, clockwise: Olle Lindvall, Åke Seiger, unknown with largely hidden face, Patrik Brundin, Håkan Widner, Ingrid Strömberg, Robert Freedman, Anders Björklund and Stig Rehn-crona. Behind the camera: Lars Olson.

ethical concerns about the use of tissue from aborted human fetuses. His decision was respected by the others in the team. Backlund was replaced by Stig Rehn-crona who was selected by the head of neurosurgery in Lund. This was an excellent choice. Stig was not only a very experienced neurosurgeon but also had a strong experimental background.

During this period, and after several hearings and discussions, guidelines for the use of human fetal tissue for transplantation purposes were adopted by the Swedish Society of Medicine in March 1986. This was a very important development. Together with approval from local ethical committees it was now possible both to perform the necessary preclinical studies with human tissue grafted into animal models and to move forward to the clinic.

One particularly important planning meeting had taken place in London in June, 1986, when Anders and I met with the leading movement disorder specialist in the world, David Marsden from Institute of Psychiatry, and with the imaging expert, Richard Frackowiak, from Hammersmith Hospital. The aim was to discuss the possibility to detect a surviving dopaminergic graft in the brain of a living

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Parkinson's disease patient. We regarded this to be crucial to understand what was happening after the implantation of fetal tissue and to clarify mechanisms of a possible improvement. Would available methods have the necessary resolution and sensitivity to demonstrate survival of grafted dopaminergic neurons? Anything like this had never been tried before. The conclusion from the meeting was that there might be a chance that a surviving graft would be detected by positron emission tomography (PET) using [^{18}F]-fluoro-dopa as tracer.

In September 1987, together with my research nurse, Lene Mangalanayagam, I traveled with the first two patients and their husbands to London for preoperative PET scans (Figure 14). The journey had to be very well prepared since both patients were periodically severely disabled in "off" phase, and could not walk at all. Ambulance personnel were waiting at the hovercraft terminal in Malmö, prepared to carry the patients on board the vessel. However, both patients were in "on" phase and perfectly mobile. When we arrived at Copenhagen airport, an ambulance was waiting to bring the patients to the terminal but both patients ran into the bus. And so it continued. Our patients had no problem entering the plane. An air hostess came up to my seat, on the second row, and asked me if I was the neurologist having two very handicapped patients with me. She looked very surprised when I pointed to the two patients who were both moving completely normally. We enjoyed the flight very much. One of our teenage-idols, Cliff Richard,

was sitting in the first row, and we started to talk to him and got autographs. Everything went very smoothly until 5 minutes before we landed at Heathrow Airport when both patients turned into a severe "off" phase and had to be carried off the plane.

We stayed for the first time at Bryanston Court Hotel, Great Cumberland Place, which for many years was to be the home for me, accompanying persons and patients when performing evaluations in London. The days were shared between PET scans at Hammersmith Hospital and



Figure 14. Olle Lindvall, Anders Björklund, two patients and their relatives at the airline terminal in Malmö, departing for preoperative PET scanning in London in September, 1987.

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neurophysiological examinations at the National Institute for Neurology and Neurosurgery, Queen Square. After Lene left the transplantation team, I was accompanied by my close colleague Per Odin and later by my very talented PhD student Peter Hagell when traveling with patients. These visits to London were highly rewarding. First, it was a very special experience for us to live close to these severely disabled patients and to become aware of all the problems they encountered. We were impressed by their courage and willingness to participate in these highly experimental studies. Second, we got the opportunity to interact directly with excellent scientists and clinicians at the Hammersmith Hospital, e.g., David Brooks, Guy Sawle, Paul Morrish, and Paola Piccini, and at the National Hospital Niall Quinn, Gregor Wenning, John Rothwell, and David Marsden. Every time I was in London with patients, I got 45 minutes audience with David, the charismatic head of the clinic, who was extremely inspiring and the most brilliant scientist and clinician I have met during my career.

In order to avoid all movements during the PET scanning, the patient's head was placed in a helmet which had been specially made for that person (Figure 15). Then the patient had to lie down on his/her back for several hours without having taken any antiparkinsonian medication. It was very important that we got as much data as possible. This was very tough for these severely disabled patients who were really heroes! When the first scanning had been completed, the head of the unit, Richard Frackowiak opened a bottle of champagne and we all cheered and were very happy. However, during the night, much of the data was lost due to computer failure. Richard became furious.



Figure 15. Patient and staff at the Hammersmith Hospital PET scan unit, prior to transplantation, September 1987. Left to right: Richard Frackowiak (the PET scan unit chief), three technicians and nurses, then Klaus (Nico) Leenders who performed the PET scans. The patient wears the special helmet that prevents her from moving during the scan. The PET scan machine is seen in the background.

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For the first two transplantations, we decided to have tissue from Karolinska Institute as back-up. The tissue from Stockholm had to be transported by air in the morning of patient surgery.

For the clinical transplantations, we had created a team with complementary expertise: Stig Rehnecrona (neurosurgery). Björn Gustavii (gynecology). Patrik Brundin (tissue dissection). Håkan Widner (neurology, immunology), and Tore Lindholm (immuno suppression). The transplantation sessions in the series of 18 patients operated in Lund followed a similar schedule. A few days prior to surgery, I was informed by Björn Gustavii about the prognosis how many women with fetuses of appropriate age were willing to donate tissue. Women were tested for diseases like HIV and hepatitis. If the number of donors seemed to be sufficient, making it likely that we would get sufficient amount of tissue for transplantation, the Parkinson's disease patient was contacted and surgery was prepared. Unfortunately, we had to cancel many times due to too little tissue. For one English patient, transplantations were cancelled 13 times!

Patients were taken into the ward on the day before transplantation and met with Stig Rehnecrona in the evening. In the morning, everyone in the team had their different tasks (Figure 16). Immunosuppressive treatment was initiated by Håkan Widner. Patrik Brundin was preparing dissections. I was in gynecology and monitored all abortions taking place, checking that the tissue could



Figure 16. November 10th, 1987: Left: Patrik Brundin and Anders Björklund preparing fetal tissue for grafting into a Parkinson's disease patient, the first in Lund using fetal tissue. Right: Neurosurgeon Stig Rehnecrona and others in action in the operating room.

be used for implantation. Stig put on the stereotactic frame, performed an MRI scan, and planned the implantation coordinates while the patient was waiting. When we had got tissue from a certain number of donors, I contacted Stig and the surgeries could start. Before implantation, Stig, Patrik, Håkan and I had a discussion about the number of donors we should use and how the tissue should be distributed in the striatum. In the surgery room, the suspension with clumps of mesencephalic tissue was sucked up into the implantation instrument by Stig and Patrik. Tissue from several donors was then injected along 2-5 tracts in the putamen and sometimes also along 0-2 tracts in the head of the caudate nucleus (Figure 17). No major adverse effects related to surgery were observed except some transient confusion in two patients who were operated bilaterally in the same surgical session.

The first two patients were operated on November 10 and December 8, 1987. The surgeries went well and the patients were monitored extensively including PET scanning at 6 months. We had decided that the first report of the outcome of the transplantations should be given at the International Parkinson symposium in Jerusalem in 1988. Nothing should leak out earlier. Before going to Jerusalem, I went to a meeting in Chicago and described the technique that we had used and everyone asked about our findings but I revealed nothing. At the same meeting, the outcome of a large number of adrenal medulla transplantations performed in the US was reported and it was clear that the findings of Madrazo could not be reproduced. The meeting in Jerusalem approached and the interest from media regarding our findings was very high. However, when we analyzed our findings from the first postoperative 6 months, both the clinical assessment and the PET scans showed minimal if any improvement and no real evidence of graft survival. In the audience, which was crowded, there was a film crew from BBC, so my presentation, describing lack of improvement, was actually filmed. I was quite disappointed but the reaction to

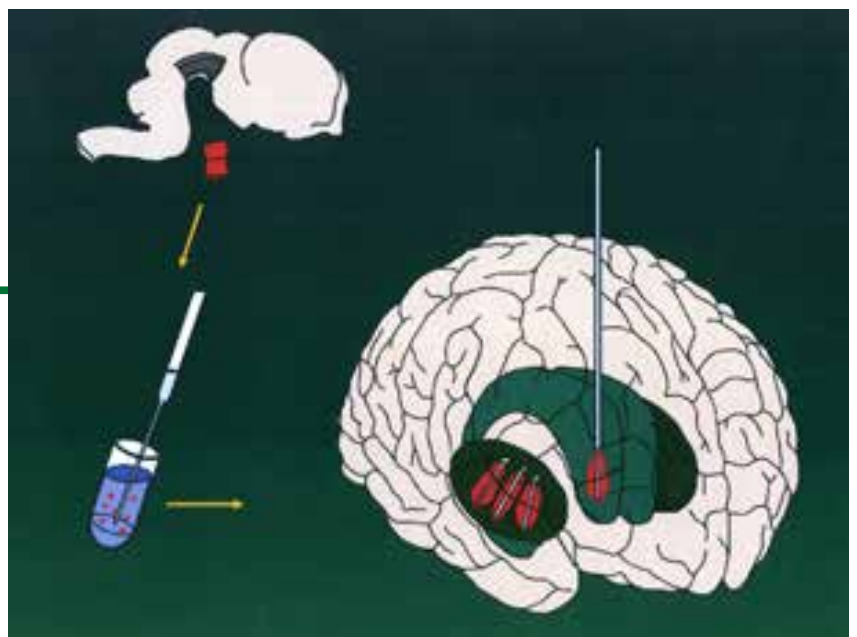


Figure 17. Schematic drawing, showing the transplantation procedure. Tissue is taken from the ventral midbrain of fetuses, dispersed in a test tube and then injected into the patient's brain at precisely predetermined sites.

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Figure 18. The Swedish dailies "Svenska Dagbladet" and "Sydsvenska Dagbladet" reported favorably about the operations already in November 1987 and January 1988, long before significant clinical results were available.

my talk was surprisingly positive, emphasizing how carefully we had performed the study and how well it was based on preclinical studies.

But we had to improve the transplantation procedure to be able to proceed. Two new patients were selected. Importantly, preclinical studies in rats performed by Patrik Brundin demonstrated that the survival of grafted human cells was influenced by the size of the implantation experiment. Based on these findings, we decided to move forward with the clinical program. Patient 3 and 4 were transplan-

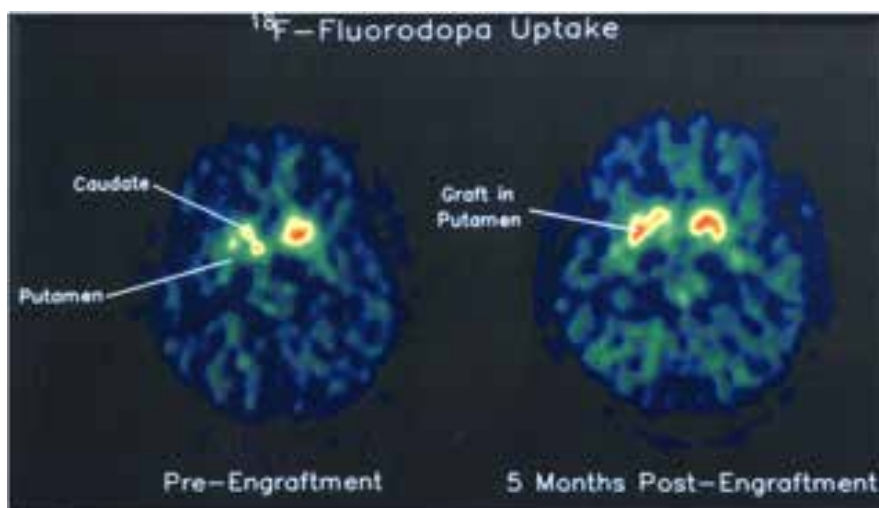


Figure 19. PET scans with ^{18}F -fluorodopa in the first patient transplanted in Lund with fetal nerve cells showed that grafted neurons can survive, in this case 5 months (and then many years more). The ^{18}F -fluorodopa signal has increased significantly in the left putamen of the patient.

ted unilaterally in the putamen on April 18 and May 26, 1989, this time using a thinner transplantation cannula.

"A brain transplant that works"

The patients were monitored every week during the whole summer. Once a month, I performed our L-dopa test. In patient 3, it was clear that something started to happen from about three months after transplantation. The movements in his limb contralateral to the grafts were faster and his rigidity was less. His condition in the morning after drug withdrawal over night was improved. The effect of one L-dopa dose lasted longer. At five months after transplantation, we decided that we had to check whether the improvement could be due to surviving graft in the patient's brain. This time Anders joined the patient and me for PET scanning in London. Already when we were in London, the preliminary images suggested that dopaminergic grafts had survived. We were extremely excited but could not believe that this was true. When I came home to Lund, I did not dare to tell my wife. After a few days we got confirmation that the PET images really showed a surviving graft (Figure 19). This finding, that grafted cells can survive transplantation into the brain of a 50 year old human affected by a chronic neurodegenerative disorder and give rise to a measurable im-

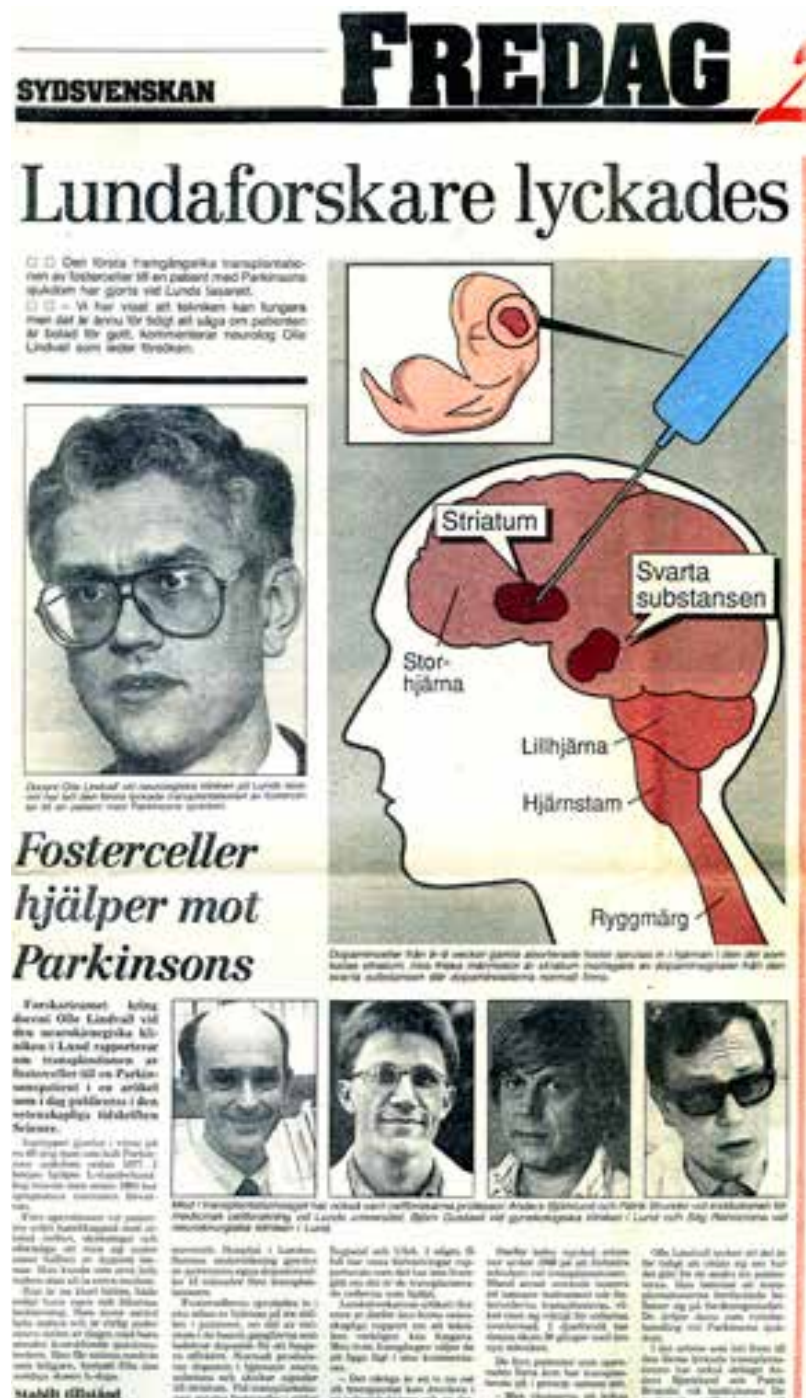


Figure 20. The Swedish daily "Syd-svenska Dagbladet" on February 2nd, 1990, reported that scientists in Lund had reached significant clinical results, and showed portraits of Olle Lindvall, Anders Björklund, Patrik Brundin, Björn Gustavii and Stig Rehncrona.

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Figure 21. The transplantations were also reported on the front page of "Dagens Nyheter", probably the most influential Swedish daily newspaper.

provement, was the most exciting ever in my scientific career. When our paper was published in Science on February 2, 1990 (247: 574-577), it caused an international media interest which none had anticipated. (Figures 20-21). Already before the embargo had been lifted, in the evening of February 1, Swedish television mentioned our study in the news program. In the morning of February 2, I was interviewed live on Canadian radio. The local newspaper "Sydsvenska Dagbladet" wrote "Lund scientists succeeded." CNN reported about our study

and articles were also published in New York Times and Washington Post. Most importantly, though, an editorial in The Lancet (1990; 335, 445-446) carried the headline "A brain transplant that works." We had reached a first and very important scientific goal.

Dopamine release from grafts

During one of the visits that Peter Hagell and I had made to Hammersmith Hospital with patients in 1998, we heard from Paola Piccini that one of her colleagues at Hammersmith Hospital had been able to quantify dopamine release in vivo in the brain of a normal subject playing a video game using PET. The idea directly came up that maybe it would be possible to measure dopamine release also from grafts. We had a unique patient who had been grafted only on the right side of the brain. He seemed perfect for the study because the non-operated side could serve as control. When Peter and I came home, we immediately prepared an application to the ethical committee. Nobody else in the transplantation team was informed. Even if I was excited about this study, I was quite pessimistic and did not think it would work. When our application had been approved by the ethical committee, I called the patient (no. 4 in our series) and explained what we were planning to do. He was very enthusiastic and immediately accepted to participate. He had done very well after surgery, been able to withdraw his L-dopa

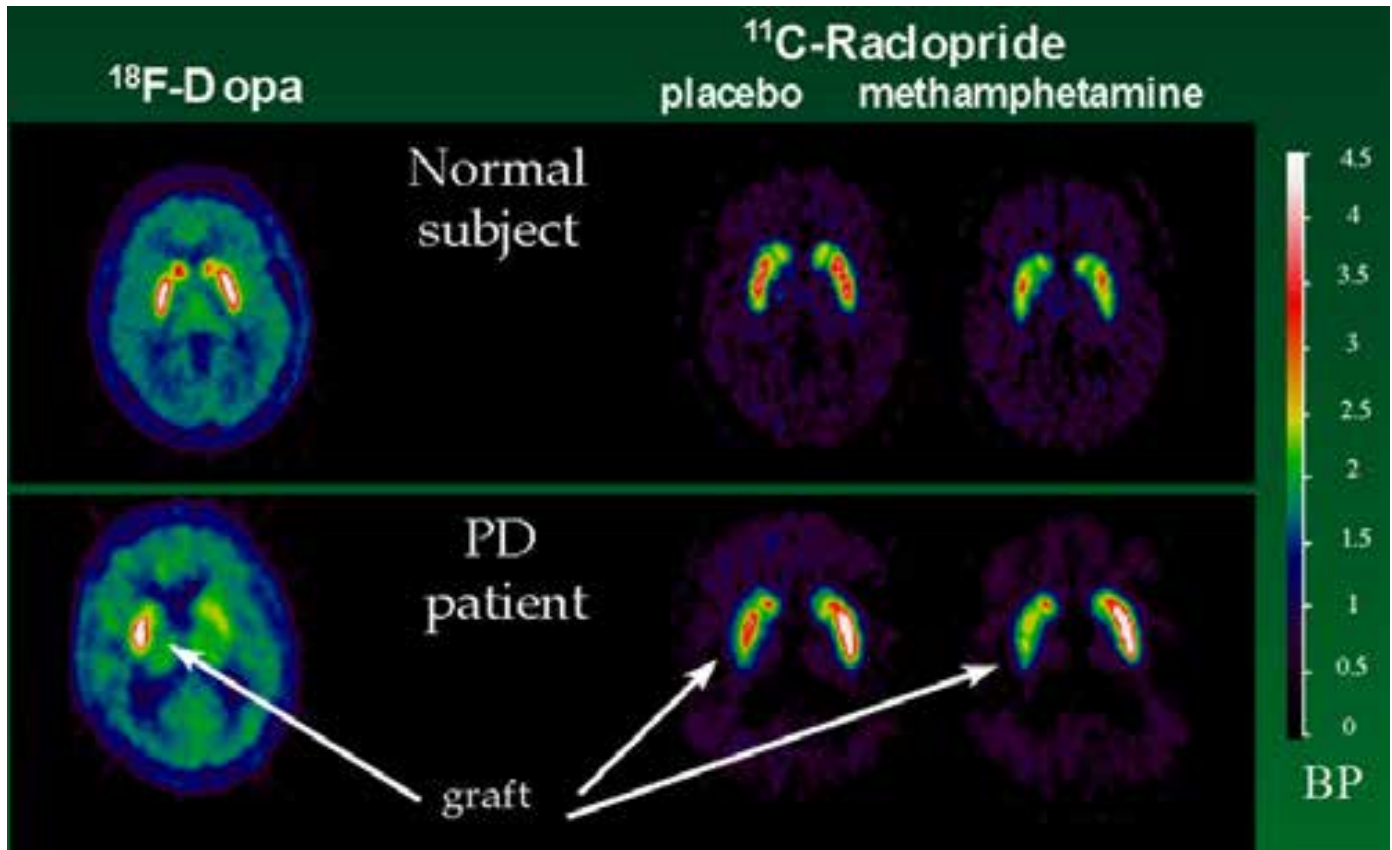


Figure 22. Tests with ^{11}C Raclopride showed that the transplants had restored dopamine release 10 years after transplantation. The patient enjoyed a major clinical improvement after the transplantation, which had allowed his L-dopa medication to be withdrawn.

treatment after three years and now, 10 years after transplantation, had only minor parkinsonian symptoms. So we left for London and the patient was subjected to two scans with the tracer raclopride. In one of the scans, amphetamine was first given to induce release of dopamine from the endogenous or grafted cells. In the other scan, saline was given. The degree of binding of the tracer revealed whether dopamine was released or not. High binding of raclopride meant low release and low binding high release. One evening I had dinner with the patient in a London restaurant. It was quite an experience to have dinner, after a walk, with this patient at 10 years after transplantation and several years after L-dopa withdrawal; he had been virtually immobile when L-dopa was withdrawn before surgery. After we had returned to Lund, I did not hear anything from Paola and I was convinced that the study had failed. When I called her after several weeks, she said that the outcome was exactly as in the best scenario: the grafts had restored both spontaneous and drug-induced dopamine release in the putamen on the operated side to normal levels. In contrast, dopamine release on the non-operated side was only 10% of normal (Figure 22). Everything had worked!! Our article was rapidly published in *Nature Neuroscience* (1999; 2, 1137-1140) and we even

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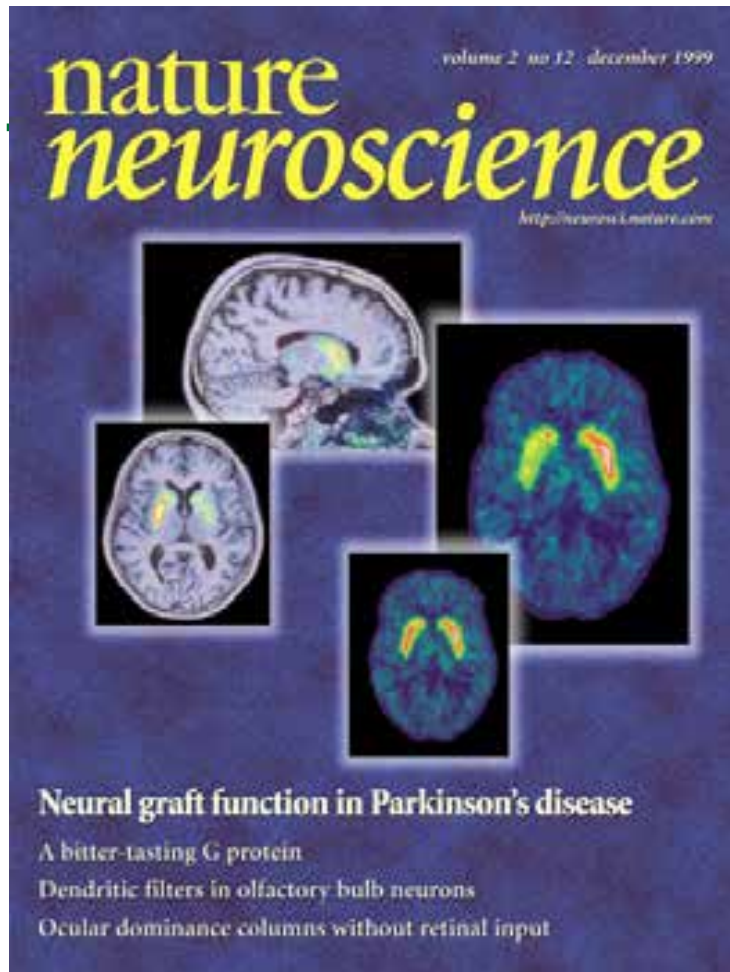


Figure 23. Cover images on Nature Neuroscience, December 1999

got our images on the cover of the issue (Figure 23).

We then used raclopride scanning to measure dopamine release from grafts in several patients. One memorable moment in connection with such a scan occurred in 1999. One patient who had undergone scan after being given amphetamine wanted to visit Selfridge's for some shopping before leaving for Sweden. He himself did not know if he had been given saline or amphetamine. When we entered the warehouse, we heard on the loudspeaker that Monica Lewinsky was going to sign her memoirs, which included a description of her relationship with the American president. I did not find it interesting but

the patient absolutely wanted to see her. We went to the floor where she was going to sign her book. The floor was crowded with people including much security personnel. The patient and I were standing in the back. When Monica Lewinsky arrived the patient became very dyskinetic with large involuntary movements in body and extremities. He rushed to take a photograph of her as close as possible. Suddenly I realized that the patient could be taken by security and they would find traces of amphetamine. He would tell security that the responsible neurologist was standing there in the background. Fortunately, nothing happened and the patient got excellent photographs of the event.

The problem with graft-induced dyskinesias

During spring 2000, I got information from a colleague in US that troublesome dyskinesias had been observed in some grafted American patients. I became very worried because of the rumors that the dyskinesias were intractable and called "run-away-dyskinesias." My worst suspicions were confirmed in June 2000, at the Movement Disorder Society meeting in Barcelona, when one of the most influential and respected movement disorder specialists in the world, Professor Stanley Fahn from New York, reported about the dyskinesias and very modest

improvements in one of the American studies. He ended his plenary lecture by saying, in front of at least 1000 movement disorder experts, "If I would have known what I know today, I would never have started with fetal transplants". This was a serious blow for the transplantation approach, which sent shock-waves through both the clinical and scientific community. We had not observed the severe dyskinesias reported by the American groups but when Peter Hagell reassessed our patients, we could observe less severe dyskinesias also in our group of patients (Nature Neuroscience 5: 627-628, 2002). It was clear that these were not due to excessive release of dopamine from the grafts as originally suspected. In a major advancement we found together with Paola Piccini and her very talented colleague Marios Politis at Hammersmith Hospital that graft-derived serotonergic hyperinnervation of the striatum was linked to the development of dyskinesias, and that they could be abolished by peroral administration of a 5-HT_{1A} receptor agonist, which dampens transmitter release from serotonergic neurons (Science Translational Medicine 2: 38ra46, 2010). Our data indicate that in future clinical trials, the serotonergic neurons contaminating dopaminergic neuron populations should be kept to a minimum or removed by cell sorting.

What influences the outcome after transplantation?

Whereas several open-label studies including our own had reported improvements after transplantation, two sham surgery-controlled clinical trials in US (published in 2001 and 2003, respectively) revealed no or very modest clinical benefit. The lack of efficacy in these studies and the occurrence of the graft-induced dyskinesias (see above) stopped clinical activities with cell therapy for a decade. Several arguments were voiced about why dopaminergic cell transplantation using human fetal mesencephalic tissue or stem cells will never be of clinical use and should be abandoned. In my view, the correct scientific strategy when the disappointing American studies were published was not to give up but to accelerate the efforts in identifying the mechanisms underlying graft-induced dyskinesias and how the motor improvement can be enhanced.

In line with this idea, Peter Hagell and I wanted to explore why some patients improved much more than others. For example, in a group of 8 of our patients, three showed excellent recovery whereas three patients exhibited no or very modest improvement. Two patients improved moderately. Surprisingly, all eight patients had surviving bilateral grafts. How was this possible? We hypothesized that the different outcome might be due to the extension of the dopaminergic denervation to areas not reached by the graft. Peter and I scored the overall outcome of the transplantations whereas Paola Piccini and her colleague Nicola Pavese independently determined the extent of dopaminergic denervation outside the grafted

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putamen in each patient. When we compared our findings, the correlation was very clear. Our findings indicated that poor outcome after transplantation is associated with progressive dopaminergic denervation in areas outside the graft (Brain 128: 2977-2986, 2005). Intrastriatal grafts should only be applied to patients with dopaminergic denervation largely restricted to striatal areas, as determined by preoperative imaging.

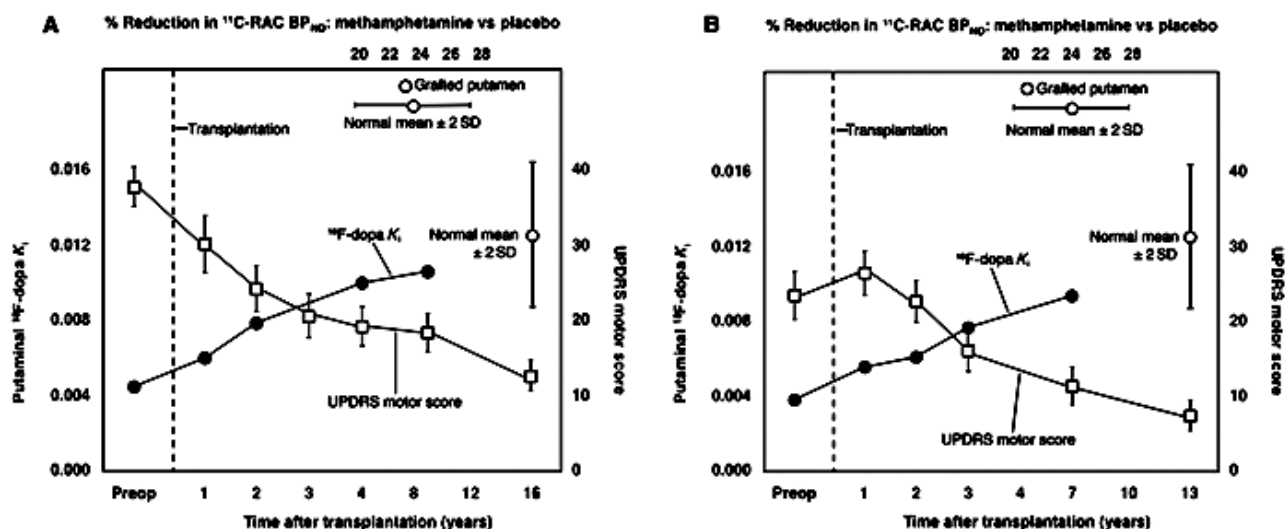


Figure 24. Fetal grafts have restored dopamine innervation and release to normal levels. They have also induced major motor improvements and made it possible to withdraw L-dopa treatment for more than 10 years in two patients with Parkinson's disease.

Can grafts survive and function long-term?

The strength of our program is that, together with our close colleagues in England and Germany, we have been able to monitor clinically and with PET the long-term outcome after transplantation. All patients have been grafted in Lund but some of them have been selected and followed postoperatively in London, by Gregor Wenning and Niall Quinn, and in Marburg/Munich by Oliver Pogarell and Wolfgang Oertel. Before we started our program, we agreed that 5 years of functioning graft with beneficial effects on symptomatology would make the transplantation approach clinically valuable. Of our 18 patients, the four most successful cases have had L-dopa treatment withdrawn, exhibiting major recovery for many years. Two patients (Figure 24) have improved so that at 16 and 13 years after bilateral intrastriatal transplantation of human fetal mesencephalic tissue, 26 and 24 years after diagnosis, and about 10 years after withdrawal

of dopaminergic medication, their UPDRS motor scores were around 10 (indicating very mild symptoms; UPDRS is a rating scale used to describe the severity of Parkinson's disease symptoms). Both dopaminergic neuron density and dopamine release assessed with PET had been normalized. Improvements in these patients were most likely due to the restoration of striatal dopaminergic function.

In 2007, when two of our grafted patients had died of causes unrelated to the transplantation, we wanted to examine their brains to determine whether the grafts had survived (Figure 25). This was done together with Jia-Yi Li and Patrik Brundin. We found, concomitantly with an American group, that many years after transplantation, a fraction of the grafted dopaminergic neurons (<2% at 11

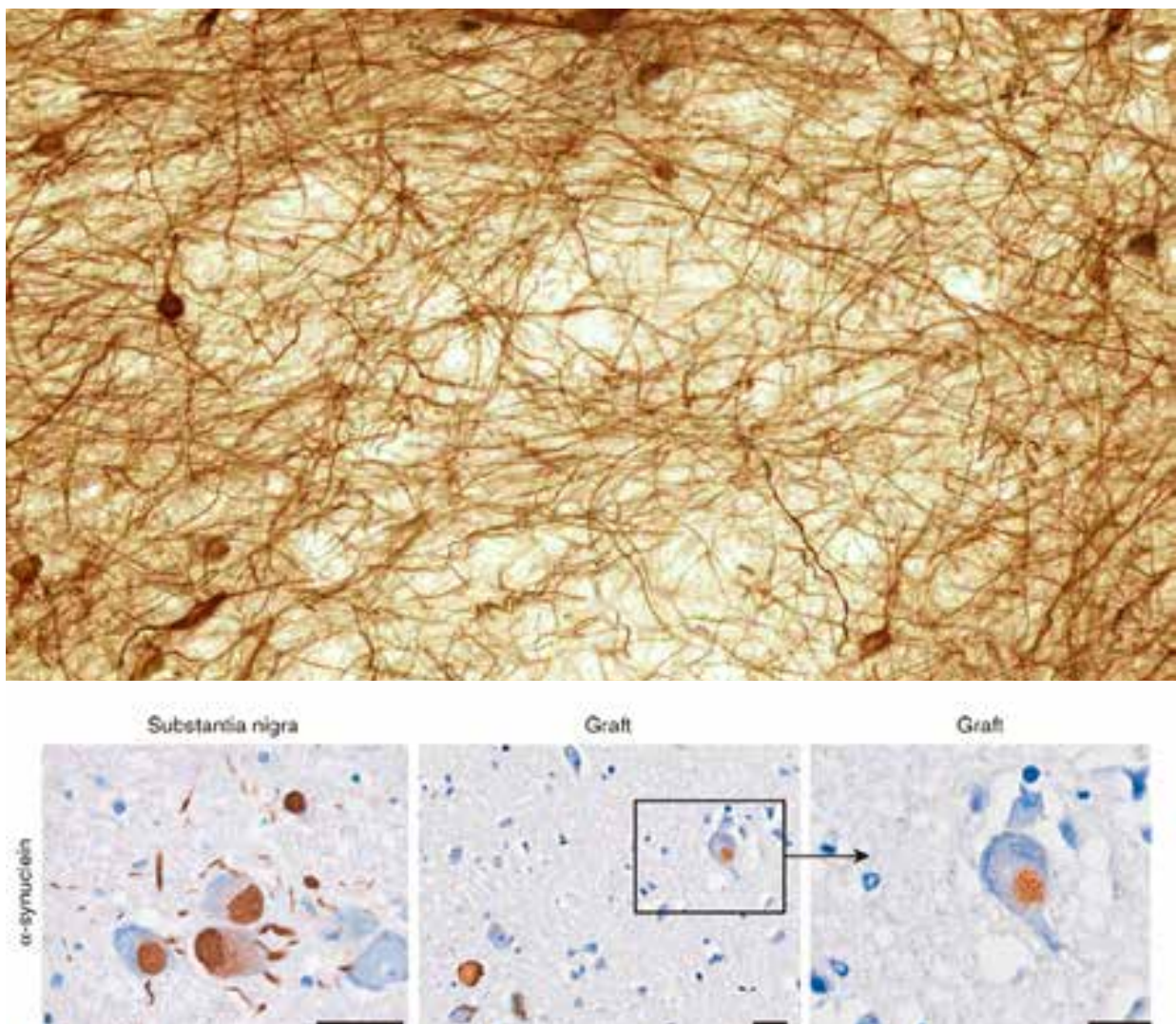


Figure 25. Histological section through the transplant in the putamen of one of the patients who died 16 years after grafting for reasons not related to his Parkinson's disease. Top: staining for dopaminergic neurons showed they had abundantly survived. Bottom: Synuclein staining (brown), showing that Lewy bodies were present in a fraction of the dopaminergic neurons, suggesting host-to-graft disease propagation.

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years and 5% at 16 years post-implantation) contained Lewy bodies, the hallmark of Parkinson's disease (Nature Medicine 14: 501-503, 2008). These findings were unexpected and opened up a completely new research field. Our data suggested that the disease can propagate from the host to the graft cells and have triggered a lot of interest in the pathogenesis of Parkinson's disease and even suggested a prion-like mechanism. However, do these findings argue against a continuation of the clinical transplantation program? In my view they do not, and dopaminergic cell therapy is still a viable therapeutic option because (i) disease propagation is slow, (ii) a majority of grafted neurons are unaffected after a decade, and (iii) patients can experience long-term improvement.

When the clinical transplantation program was initiated, Parkinson's disease was mostly regarded as a motor disorder but it is now well established that it comprises also a series of non-motor symptoms. By implanting dopaminergic cells into putamen, non-motor symptoms originating from impairments outside striatum or in non-dopaminergic systems are unlikely to be alleviated. Together with Marios Politis and Paola Piccini, we studied three Parkinson's disease patients at 13-16 years after intrastriatal transplantation of human fetal mesencephalic tissue. Dopaminergic innervation was normalized in the basal ganglia associated with major relief of motor symptoms. However, the patients developed non-motor symptoms, e.g., fatigue, anxiety, mood swings, and sleep problems. A serotonergic neuron marker was reduced in raphe nuclei and regions receiving serotonergic projections (Science Translational Medicine 4: 128ra141, 2012). Most likely the serotonergic degeneration underlies some non-motor symptoms in these patients.

These findings also illustrate that intrastriatal dopaminergic cell therapy will never provide a cure for Parkinson's disease. Importantly, though, the three patients with well-functioning dopaminergic grafts but continuous serotonergic denervation in the forebrain have declared that the advantages with the motor improvements outweigh the worsening of non-motor symptoms. I would argue that if dopaminergic grafts induce major relief of motor symptoms for more than a decade, this approach will be clinically competitive despite occurrence of non-motor symptoms.

Perspectives

Anders Björklund and Olle Lindvall

As we have tried to describe here by sharing our personal recollections, developing cell transplantation for Parkinson's disease has been a long and winding journey. The most important outcome of this research has probably been to provide proof-of-concept for the cell therapy approach in human brain disease by de-

Figure 28. Attentive listeners in the audience.

monstrating that neuroblasts transplanted into the brains of 50-60 years old patients with a neurodegenerative disease can survive for more than a decade, provide a local reinnervation, become functionally integrated in host neural circuitries, and release transmitter, thereby replacing those cells which have died. One of us (OL) remembers that when he started in clinical neurology in the mid 70-ies, the head of the clinic advised him to

leave the research on strategies to repair the brain immediately because it had no relevance for human disease. "Everyone knows that repairing the human brain is impossible." Since then, research in Parkinson's disease has contributed to completely changing our view on the plasticity and regenerative capacity of the human brain. However, the cell transplantation procedures which were tested from late 1980-ies until about 10 years ago did not provide clinically competitive treatment in Parkinson's disease and, moreover, some grafted patients developed troublesome off-medication dyskinesias.

Although Parkinson's disease patients now have more therapeutic options than 30 years ago, a treatment giving rise to robust, long-lasting improvement of motor function is still highly warranted. Several recent scientific advancements are particularly important and supportive of the further development of a cell therapy for the ailment. Factors determining the outcome after transplantation in Parkinson's disease patients, such as preoperative denervation patterns, have been identified. Mechanisms of graft-induced dyskinesias, especially the role of serotonergic neurons, are much better understood, and this adverse effect can be treated and most likely also prevented. In the ongoing EU-sponsored TRANS-EURO clinical trial, in which Lund University is a partner, human fetal mesencephalic tissue will be implanted into striatum with optimized patient selection and tissue preparation procedures. Although this trial is likely to give valuable information, TRANSEURO will not, due to the scarcity of human fetal tissue, solve the problem of the need for large quantities of dopaminergic cells for implantation. Importantly, dopaminergic neurons can now be generated from stem cells in large numbers and standardized preparations, and are soon ready for clinical application. In our view, these new preclinical and clinical data justify moving forward in a responsible way with the development of a dopaminergic cell therapy in PD, which should be tested in well-controlled clinical trials.



Photo: Per Lindström