Anders Björklund

Born:

Söderhamn, Sweden
July 11, 1945

Education:

Lund University, Sweden, Med. Candidate (1966)
Lund University, Sweden, MD, PhD (1969)
University of Oxford, UK, MS (1989)

Appointments:

Docent of Histology, Lund University, Sweden (1970)
Associate Professor of Histology (Lektor), Lund University, Sweden (1973)
Professor of Histology, Lund University, Sweden (1983)
Visiting Professor at the College de France, Paris (April 1985)
Newton-Abraham Visiting Professor, University of Oxford, United Kingdom (1989–1990)
Senior Professor, Lund University, Sweden (2012)

Honors and Awards (Selected):

Doctor Honoris Causa, University of Turin, Italy (1987)
Doctor Honoris Causa, University of Copenhagen, Denmark (1989)
Member of the Royal Swedish Academy of Sciences (1989–present)
The Zulch Prize from the Max-Planck Society, Germany (1990)
The IPSEN Prize in Neuronal Plasticity, Paris (1990)
The Charles A. Dana Award from the Dana Foundation, New York (1993)
The Anders Jahre Prize for Medical Research, Oslo, Norway (1995)
The Söderberg Prize for Medical Research from Swedish Society for Medicine (2000)
The Wendell Krieg Lifetime Achievement Award from the Cajal Club (2006)
The Robert A Pritzker Prize from the Michael J Fox Foundation (2011–present)
The Eric K Fernström Nordic Prize (2011)
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Anders Björklund received his MD and PhD at Lund University in Sweden in 1969 and has spent his entire academic career at this institution. He was appointed professor of histology in 1983, and since 2012, is senior professor at the Wallenberg Neuroscience Center in Lund. He was president of the European Neuroscience Association 1996–1998 and served as chairman of the Neuroscience Panel at the European Research Council in Brussels in 2007–2010. He was elected member of the Royal Swedish Academy of Sciences in 1989, and foreign member of the National Academy of Sciences, USA, in 2011. His main research interests are in studies of brain regeneration and repair, with a focus on cell replacement and gene therapy in neurodegenerative diseases. In the 1970s, his team pioneered the development of methods for cell transplantation to the brain, and over the following decades, Björklund's team at Lund University has pioneered the use of cell transplantation in Parkinson's disease and has played an active role in the exploration of stem cells as a source of cells to be used for this purpose.

Anders Björklund

As a scientist I have been passionately curiosity driven, always looking forward to the next experiment and exploring new ideas. This has made me, I believe, more interested in what is ahead of me rather than recording the events as they happen. I have been poor at taking notes of events and I have kept no diary, so when I now endeavor to recall how my research has evolved over the years, I have had to resort to my notoriously imprecise memory and the support I have found in my correspondence, which, before the Internet era, I had collected in 60 thick files.

Looking back, I realize that this lack of detailed written records has helped me to retain the positive memories and the fascination and excitement embedded in the pursuit of new ideas and the exploration of the unknown. And my luck was that I entered brain research at the time, in the late 1960s, when modern neuroscience was born, and in the laboratory where a new groundbreaking microscopic technique, the Falck-Hillarp monoamine histofluorescence method, had just been developed. It was like walking into an open landscape: Everything was new, and everything was possible. Memories of failed experiments, rejected papers, and frustrating struggles for grant money all seem to kindly fade away with time. And wisely were not kept on record.

My research career has been unadventurous in the sense that I have remained at the same institution, Lund University in Sweden, where I started my medical studies in 1964. I was drawn into research already during my second year in medical school, and full of enthusiasm I finished my hybrid MD/PhD thesis in two and a half years. I was then 24 years old and had to decide whether I wanted to complete my medical training or stay in research. For a few years, I entertained the possibility to go abroad on a postdoc, but the support and the laboratory resources provided by my mentor Bengt Falck made it less attractive to leave Lund, particularly since Bengt generously gave me the freedom to develop my own ideas and pursue my own research. It was unlikely that I could find similar opportunities elsewhere. And over time, I have become very fond of Lund—a small, practical, and very friendly town that combines the very old with the very new, dominated by the university and its lively student activities. A good place for a compulsive thinker like me! Instead of moving elsewhere I have been fortunate to spend three stimulating sabbaticals abroad, with my family. Twice with Susan Iversen and Steve Dunnett in Cambridge, United Kingdom, and once with Paul Bolam and Peter Somogyi in Oxford. In retrospect, it is clear that these visits were important and given me opportunities not only to "recharge my batteries" but also to reflect and find new directions for my research.

Early Days

I think it was inevitable that I should end up as an academic. My parents were both university educated, at Uppsala University, and it was there they met and formed a family. My father studied to become a clergyman, but he soon realized that a job in the Swedish state church was not for him. So after just a couple of years as a clergy, he decided to return to Uppsala to complete his licentiate degree and become a high school teacher in philosophy and religion. My mother also studied to become a teacher, in English and Swedish literature, and their first jobs were in a small town north of Uppsala, Söderhamn, where I was born, three years after my older brother. A year later, we moved further north to Boden, a town close to the pole circle where a new gymnasium (high school) had just opened, offering them both good teaching jobs. Boden was at the time known for housing four military regiments (all gone now), and thus was full of young men completing their (compulsory) military service. When I turned eight, my parents had had enough of the cold and dark winters up north and moved to Växjö, a bigger town centrally located in southern Sweden, known for its cathedral and as a bishop's site, and with an old cathedral school where the famous botanist Carl Linnaeus had spent his formative years.

It was here, in Växjö, that I went to school and finished my *studentexamen* (high school exam). Like my older brother, I was an exemplary student. My favorite subjects were physics and math. I had superb and engaged teachers and was initially convinced that I wanted to study these subjects also at the university. However, the fact that several of my classmates planned to apply to medical school made me rethink my plan. So to keep this option open, I had to add biology as my extra subject in my last year, a necessary complement if I wanted to go to medical school. The cathedral school in Växjö was full of highly qualified teachers with the ability to stimulate and engage the students, and my biology teacher was one of them. I realized that studies of living matter were very special and, at this time, a decade after the discovery of the double helix, it became clear to me that biology had become an exciting subject.

How It Started

I began my medical studies in Lund in 1964. Lodging in the student dormitories was difficult to find for the newcomers, so for the first two years, I rented a room from a lovely octogenarian widow who treated me with tea and sandwiches on a tray in my room on Sunday mornings. There I stayed until I found my own small apartment, with bathroom and a tiny kitchenette, conveniently located above a fine gourmet shop on one of the main shopping streets, just a 10 minute walk from the Histology Department where I now was employed as part-time teaching assistant, *amanuens* as it

was called. There were three of us, and our job was to be present during the microscopy lectures, run the microscopy projector, and assist the students at the microscope and in the lab practicals. Rather boring but the salary, though small (250 SEK, around US$20, per month at that time) was a good addition to the state-guaranteed student loan that had just been introduced.

The reason I ended up in histology was twofold: I enjoyed doing microscopy and found that I was quite good at it. And in the histology course, the associate professor Bengt Falck had given a lecture on a topic that had intrigued me: a new microscopic method that allowed visualization of neurotransmitters in microscope sections. At the end of the lecture, Bengt said that he would welcome any interested student to visit his lab. In the following break, I persuaded one of my fellow students, another Anders, to give it a try. We were the only two in the class to turn up, and Bengt told us that we were welcome to follow the work in the lab and try our hands on the new method in our spare time. Spare time was no problem for me, but for the other Anders "spare time" soon disappeared when he became the father of twins.

The new method, now named the Falck-Hillarp histofluorescence method, had been developed a few years earlier by Bengt and his mentor and senior colleague Nils-Åke Hillarp. Bengt and Nils-Åke had been exploring various ideas on how to visualize noradrenaline in the microscope and the one that finally worked was based on the use of hot gaseous formaldehyde to convert the nonfluorescent catecholamines to fluorescent tetrahydroisoquinolines that could be detected under blue-violet light in a fluorescence microscope, shining with a yellow-green color. The method was published in 1962 and at the time when I walked into Bengt's lab, in 1965, it was clear that it had opened tremendous opportunities, not only for Bengt and his collaborators, but also for the lab at the Karolinska Institute in Stockholm that Hillarp had started when he moved there as professor in 1962. I learned that Hillarp had sadly died from a malignant melanoma, but in the short time he was active in Stockholm, he had assembled a tremendous team of young students and collaborators, including Kjell Fuxe, Annica Dahlström, Gösta Jonsson, Urban Ungerstedt, and Lars Olson, who all went on to make important contributions to the budding field of monoamine research.

Looking back, it is clear that the F-H method came to play a very important role in the study of monoamine neurotransmitters, dopamine, noradrenaline and serotonin. It made possible, for the first time, the anatomical mapping of the monoamine systems in the nervous system, not only in mammals but across species, and it opened entirely new opportunities for pharmacological and functional studies of dopaminergic, noradrenergic, and serotonergic neurotransmission in both the central nervous system (CNS) and PNS. It dominated the field for about two decades, until the mid-1980s, when immunohistochemistry took the lead. During that time the Swedish labs in Lund, Stockholm, and Göteborg remained at the forefront, driving the technical development and setting the standards for how it should

be applied. The fact that it was technically tricky worked against it being more widely spread. The method used a freeze-drying procedure to remove water from the tissue blocks. This step was capricious and could easily go wrong, and it seemed that it did not work well in warm and more humid climates, such as—we were told—in Floyd Bloom's lab in Washington. Our cold climate apparently played to our advantage in this case.

I was lucky to join Bengt's lab at the time when the method was in full swing and the opportunities were endless. The lab was housed in a modest dwelling, a wooden annex to the main department and because of its color popularly known as "the pink barrack." Bengt's main interest at this time was in endocrinology and reproduction, and he had a close collaboration with a clever zoologist, Anders Enemar, who was engaged in a project to describe the development of the catecholamine system in the hypothalamo-pituitary system in the mouse. I rapidly got on friendly terms with him, and he invited me to join as a collaborator in this project. At the same time Bengt suggested that I should take on a project that involved measurements of dopamine and noradrenaline levels in the different subportions of the pituitary (the anterior, posterior, and intermediate lobes) and the hypothalamic median eminence. This could not be easily done in rats and mice, but he had good contacts with the local slaughterhouse where they regularly extracted pituitaries from pigs (delivered to a local pharmaceutical company for extraction of human pituitary hormones). Bengt arranged for me to go there early in the morning, before my lectures started, to collect the tissues. His lab had an old mini-truck that I could use to get there. So once a week, I went up early in the morning, drove the 15 km to Kävlinge, collected the dissected tissue in tubes frozen in dry ice, delivered them to the Evald Rosengren's biochemistry lab in pharmacology, and then off to the start of the first lecture of the day. I mostly made it, as I recall!

Years earlier Evald, then a doctoral student in Arvid Carlsson's department, had published the first description of the regional distribution of dopamine in the brain. This study had been published together with his fellow MD student Åke Bertler in the Swiss journal *Experientia* in 1959, less than a year after Carlsson's seminal *Science* paper on the presence of dopamine in the brain. They found that dopamine was highly concentrated in the striatum, which led them to conclude that

> the results favour the assumption that dopamine is concerned with the function of the corpus striatum and thus with the control of motor function. This view is supported by the fact that drugs which influence the dopamine content of the striatum also produce disturbances of motor activity. . . . Excess of dopamine in the brain produced by administration of DOPA is accompanied by motor hyperactivity. (Bertler and Rosengren 1959)

As described in his autobiographical chapter in volume 4 of this series (Hornykiewicz 2004), it was this paper that triggered Oleh Hornykiewicz, a young pharmacologist in Vienna, to initiate his classic postmortem studies of dopamine in brains of patients with Parkinson's disease (PD). After his dissertation in 1960 Evald moved with Arvid Carlsson to Göteborg and returned a few years later to a professorship in Lund where he initiated a fruitful collaboration with Bengt and his team that lasted until his retirement.

This pig study led to my first scientific paper, which was submitted to *Life Sciences* in July 1967 with me as first author (Bjorklund et al. 1967; the convention at the Swedish universities at the time was to list authors in alphabetical order and the leading Scadinavian journal, *Acta Physiologica Scadinavica*, for example, had adopted this principle as a rule). Although limited in content, this eight-page article made me proud. I had been allowed to write the first draft myself, and this was my Opus 1! Emboldened, and much encouraged by Bengt, I decided to take a break in my medical studies and start as a full-time doctoral student in Bengt's lab. The biochemical data in the *Life Sciences* paper strongly suggested the presence of a dopaminergic innervation of both the posterior and intermediate lobes of the pituitary. I went on to investigate that in further detail using the F-H method, and in two follow-up papers, published in *Zeitshrift für Zellforschung* in 1968 (Bjorklund et al. 1968), and in *Brain Research* in 1970 (Bjorklund et al. 1970), I could show the presence of a dense dopamine fiber network in these parts of the pituitary, in mouse, rats, cats, and pigs, originating in the hypothalamic A12 cell group previously described by Annica Dahlstöm and Kjell Fuxe in Stockholm. Tiny in size, of course, but I had discovered a previously unrecognized dopamine projection system. Not so bad, I thought!

The Glyoxylic Acid Method and My Neuroanatomical Work

When I defended my thesis in October 1969, it was clear that, rather than going back to my medical studies, I wanted to continue in the lab. At that time, I had been appointed to an assistant teacher job, and in 1970, I had acquired my docentship, which made it possible for me to act as supervisor of new doctoral students coming to the lab. It was a fantastic time. Bengt's lab was like a hotbed, boiling with activity. It was also very well funded and offered unlimited possibilities for a young enthusiast like me. This was the place to be, I felt. Of the many young medical students and doctors working in Bengt's lab at the time, six went on to develop highly successful independent research careers, eventually ending up as professors in the faculty: Christer Owman in histology, Berndt Ehinger in ophthalmology, Nils-Otto Sjöberg in obstetrics and gynecology, Frank Sundler in histology, Olle Lindvall in neurology, and myself in histology. Thus, the creative research environment in the pink barrack fostered a whole new generation of researchers with a major and lasting impact on the development of modern neuroscience in Lund.

During my thesis work, I had become involved in the efforts to optimize and improve the F-H method. The method was quite capricious: If the freeze-dried tissue was too dry, the formaldehyde reaction would not run, and if the tissue was too wet, the catecholamines would diffuse away from their storage sites. The Karolinska group had come up with a reasonably good way to standardize the reaction, that is, to add a defined amount of water to the paraformaldehyde powder by storing it at a predetermined humidity in a sealed container. Nevertheless, it was clear that the sensitivity was not optimal: It usually allowed good detection of cell bodies and the varicose beads of axon terminals, but not of preterminal axons and dendritic processes where the levels of catecholamines and serotonin are lower. There was clearly room for improvement.

At this time Olle Lindvall, a medical student two years younger than me, had joined the lab and Bengt suggested that he join me in this work. We decided to look for an alternative and more efficient reagent and set off to perform a screen of a broad range of so-called carbonyl compounds that could be expected to react with the monoamines in a similar way as formaldehyde. In this screen, which was performed on amines enclosed in dried albumin droplets, we zoomed in on one that seemed most promising: glyoxylic acid (GA). Formaldehyde has the chemical formula H-CHO. In GA, HOOC-CHO, the hydrogen is replaced by carboxylic acid. GA reacted with catecholamines and serotonin in a similar way as formaldehyde, but the yield and brightness of the fluorophores were considerably higher, resulting in a five- to eightfold increase in sensitivity compared with the standard F-H reaction. In our first attempts we exposed tissue sections to hot GA vapor, as used in the F-H method, but the best results were obtained with tissue that had been perfused with an ice-cold 2 percent GA solution (later with 0.5 percent formaldehyde added to obtain better fixation) followed by sectioning on a Vibratome, three- to five-minute immersion of the sections in GA, and heating at 100°C for 6 minutes. This allowed microscopy of the sections within half-an-hour after perfusion. Vibratome sectioning had just been introduced by Tomas Hökfelt at Karolinska, and our first application of the GA method to Vibratome sections was thus carried out during a short visit to Tomas' lab in Stockholm, resulting in a short joint paper in *Histochemie* in 1973 (Lindvall et al. 1973).

The results obtained with the GA-Vibratome method were absolutely stunning: The fluorescence was bright and sharp against an essentially black background, axons and dendrites were visualized in their entirety, and even the tiniest of axonal braches and terminals were nicely detectable. Olle and I returned home full of excitement. We ordered first one, and soon after, two more Vibratomes and got started. It soon became clear to us that the new GA method allowed us to map the catecholamine systems in the brain with a sensitivity and precision that went far beyond what had been possible with the classic F-H method and, more important, we could detect previously

unobserved axonal pathways and projections, such as the first demonstration of the midbrain dopamine projection to neocortex, the previously unknown projection of the A13 cell group in the zona incerta, and the storage of dopamine in the dendrites of the nigral dopamine neurons, suggesting a role of dopamine in dendritic terminals. Olle and I had a great time at the microscope and enjoyed the challenge of getting all the new findings published as quickly as possible. Over a little more than a year, we reported the new findings in five papers, published in rapid succession, including a detailed description of the organization of the ascending catecholamine projection systems in the rat brain that was published as a supplement to *Acta Physiologica Scandinavica* in 1974 (Lindvall and Bjorklund 1974). This paper has had major impact in the field and, over the years, has been cited (according to Google Scholar) more than 1,300 times. It also formed the core of Olle's doctoral thesis, which he defended in May 1974.

Although very sensitive, the GA-Vibratome method was not well suited for processing of larger numbers of brains, such as in experimental studies in which the effects of interventions are compared between groups of animals. Vibratome sectioning was slow and had to be done more or less online and was less suitable for serial sectioning of larger specimens. For such studies, the F-H method was more attractive. Paraffin-embedded blocks of tissue, as used in this method, allowed bulk processing of a large number of specimens with a consistent fluorescence yield in samples processed together and facilitated storage, sectioning, and handling of the specimens. Thus, we continued our efforts to increase the sensitivity of the freeze-drying method. We focused on the fact that the formation of fluorophores in the F-H reaction is efficiently catalyzed by acids. In model experiments, we found that metal ions, like magnesium and aluminum, in particular, are efficient fluorescence-promoting agents in the formaldehyde reaction and that this effect can be explained by the fact that these metal ions are acids according to the so-called Lewis acid–base definition. In subsequent *in vivo* experiments, we could show that addition of aluminum sulfate to the formaldehyde perfusion solution markedly increased the sensitivity of the method for the detection of catecholamines by about fivefold.

The sensitivity of this improved method for the visualization of catecholamines, coined the ALFA (aluminum-formaldehyde) method, closely matched that obtained with the GA-Vibratome method. It was published in 1980 (Loren et al. 1980) and became the standard method in all our subsequent work. Using this versatile method, Olle and I, together with a talented PhD student, Gunnar Skagerberg, went on to explore and map several previously unrecognized dopamine projection systems, such as the dopaminergic projections from the diencephalic A11 cell group to the spinal cord (Skagerberg et al. 1982), the meso-habenular dopamine pathway (Skagerberg et al. 1984), and the dopaminergic innervation of the neurosecretory paraventricular and supraoptic nuclei (Lindvall et al. 1984).

The grand finale of our neuroanatomical studies, spanning over more than a decade, was the publication of two major reviews, in Volume 2 of the *Handbook of Chemical Neuroanatomy*, in 1983, and in Volume 4 of the *Handbook of Physiology*, in 1986. When I look at them now, I am very pleased: Two decades after the pioneering work of Fuxe and Dahlström, Olle and I had provided the first fully comprehensive account of the anatomy of the dopamine and noradrenaline systems that remains on the whole accurate and still valid today. And the first of the two, in particular, has been very well cited over the years.

During the 1980s, it became gradually clear to us that the immunohistochemical (IHC) techniques were going to take over. Thanks to the increasing commercial availability of antibodies suitable for use on tissue sections, the IHC method had became highly versatile and also readily available for the wider research community. For visualization of catecholamine systems, the access to excellent tyrosine hydroxylase (TH) antibodies was the turning point, and at the end, we in Lund were also converted to TH immunostaining on cryostat or freeze-microtome sections. The very last studies in which the aluminium-formaldehyde (ALFA) method was used in Lund were published in 1988 and soon thereafter the imposing freeze-dryers that had been built in Lund were dismantled after more than 25 years of continuous use! As is often the case with technologies that are superseded by new technology, the F-H method, despite its historical impact and its dominance in the field over a quarter of a century, is virtually unknown to neuroscientists active today.

Serotonin Neurotoxins

In 1967, Hans Thoenen and Jean-Pierre Tranzer, working at the Hoffman-LaRoche company in Basel, discovered the ability of the noradrenaline analogue 6-hydroxydopamine (6-OHDA) to induce an acute and selective degeneration of sympathetic noradrenergic nerve terminals. This remarkable observation was rapidly picked up by a young graduate student in Stockholm, Urban Ungerstedt, who showed that this compound could be used for selective lesioning of dopamine and noradrenaline neurons also in the brain. Two years later, in 1970, Ungerstedt and Gordon Arbuthnott in a now-classic paper introduced the unilateral 6-OHDA lesion model, combined with the amphetamine-induced rotation test, which has become a standard tool in dopamine research and is still widely used today.

It was known that 6-OHDA acted as a "false transmitter" in catecholamine neurons, meaning that it was taken up and stored by the same transport systems as dopamine and noradrenaline. Because of its oxidative properties, however, 6-OHDA is easily auto-oxidized, leading to the formation of toxic reactive oxygen species within the target cell, leading to mitochondrial damage. The selectivity of 6-OHDA for catecholamine neurons is

due to the fact that it is effectively taken up by the membrane transporter, leading to an accumulation of the drug selectively within dopamine and noradrenaline neurons. This mechanism suggested that similar neurotoxins could be developed for lesioning of the serotonin neurons in the brain.

This idea was picked up be a young anatomist in Hamburg, Hans-Georg Baumgarten. He had an ongoing collaboration with Bengt Falck using the F-H method to map the distribution of catecholamines in frogs, lizards, and lampreys, as well as in various human tissues, and I came to know him during his frequent visits to Lund. Inspired by the neurotoxic properties of 6-OHDA, and with the help of a clever chemist in Munich, H. G. Schlossberger, he arranged for the synthesis of hydroxylated derivatives of serotonin (5-hydroxy-tryptamine), and one day in 1970, he came to Lund with a sample of freshly synthesized 5-6-dihydroxy-tryptamine (5,6-DHT) and asked me if I wanted to join in the in vivo testing of this compound (Baumgarten et al. 1971). This was the start of a very productive collaboration that spanned almost a decade. With Schlossberger's help, and through other sources, Baumgarten managed in the end to get access to more than 20 5-HT analogs that we tested both in vitro and in vivo. The most promising results were obtained with 5,6-DHT and 5,7-DHT, but both had their disadvantages: 5,6-DHT induced nonspecific tissue damage at higher doses, and 5,7-DHT had a significant toxic effect on noradrenergic neurons as well. We zoomed in on 5,7-DHT as the most effective and selective compound, and we found that the noncoselective damage to noradrenaline neurons could be effectively counteracted by coadministration with a catecholamine uptake blocker, DMI. 5,7-DHT, administered either intraventricularly or directly into the brain parenchyma, has remained a standard experimental tool for selective damage of serotonin neurons (Baumgarten et al. 1973; Bjorklund et al. 1975a).

In our studies of the effects induced by the intraventricular serotonin neurotoxin lesions, we made an intriguing observation: The damage induced by these neurotoxins was primarily hitting the axons and leaving the cell bodies intact. This was particularly striking in the descending serotonin projection from the brain stem to the spinal cord, the so-called bulbo-spinal serotonin system (Bjorklund et al. 1973). In the treated animals, axonal damage appeared within the first four days after injection. These swollen and distorted axonal profiles were mostly confined to the caudal medulla oblongata and at the junction to the spinal cord and was accompanied by a nearly complete loss of serotonin terminals at all levels of the cord after two to three weeks. This was followed by a remarkably efficient regenerative response, starting about one week after injection and progressing over the subsequent weeks and months. A near-normal innervation was restored in nuclei of the lower brain stem (inferior olive and facial nucleus) within a month, and in the rostral segments of the cervical spinal cord after about three months. At longer survival times, 7–12 months postinjection, regenerated axons and

terminals also were observed further caudally, in the previously denervated thoracic segments, several centimeters below the initial axotomy (Bjorklund and Wiklund 1980; Wiklund and Bjorklund 1980).

The neurotoxin lesion apparently provided favorable conditions for efficient long-distance axon growth along the spinal cord. Compared with standard mechanical or electrolytic lesions, the regeneration of new axons in the toxin-treated rats takes place in brain tissue that is morphologically intact and in the absence of impeding necrotic tissue and scars. Strikingly, the regenerating axons were seen to re-innervate the correct terminal areas with a high degree of specificity, suggesting that the regrowth of the serotoninergic axons was controlled by precise mechanisms of guidance and recognition, a phenomenon that we were to explore in much greater detail in our cell transplantation studies.

How I Got Interested in CNS Regeneration

The studies of neurotoxin lesions were among the very first to demonstrate the ability of CNS neurons to regenerate efficiently, and over larger distances, after axotomy. My interest in central nervous system (CNS) regeneration, however, was triggered several years earlier thanks to an American neurologist, Bob Katzman, who spent a six-month sabbatical in Bengt Falck's lab in 1969. I had just finished my PhD thesis and decided to stay on in Bengt's lab and was put to work helping Bob in his experiments.

Bob wanted to find out where the therapeutically active dopamine is formed from L-DOPA when the intrinsic nigro-striatal dopamine system is removed. A young neurosurgeon, Kurt West, made electrolytic lesions unilaterally in the substantia nigra in rats, and the brains were processed for F-H fluorescence microscopy at varying time points. The localization of L-DOPA-derived dopamine turned out to be difficult to spot in the striatum. When looking at the sections through the nigra itself, we not only saw the damage caused by the lesion, and the associated loss of dopamine neurons, but also and most conspicuously a pattern of fine-caliber fluorescent fibers that was not there at short time points. They emerged over time, during the second and third week, most prominently along the border of the lesion and the electrode tracks and invading the walls of adjacent blood vessels and had all the features of regrowing noradrenaline containing axonal sprouts. This raised the possibility that they were derived from the sympathetic innervation of the meningeal vessels. They remained after sympathectomy, however, so we concluded that they must be of central origin, most probably derived from the noradrenergic axon bundle that passed through the lesioned area. This axon bundle, which originated in the locus coeruleus, was transected by the electrolytic lesion, and a closer inspection in the microscope suggested that the newly appearing delicate fibers were sprouts that had grown out from the severed axon stumps. This was one of these

unexpected, serendipitous findings that easily could have gone unnoticed, but Bob convinced me that it was really important: Regeneration was not supposed to happen in the brain!

We published this study in *Brain Research* in 1971 (Katzman et al. 1971). These findings were indeed intriguing but how could we prove that these growing axons indeed were regenerating? Here, Ulf Stenevi comes on the stage. Ulf was a friend and classmate in medical school. For some reason, he decided to take a break from his medical studies and started a PhD project in Bengt's lab. Perhaps he felt he needed to do something else for a while, or perhaps I had convinced him that lab work was fun and exciting. Anyway, when Ulf started in the lab in 1970, it was the beginning of a fantastically enjoyable and stimulating collaboration that lasted more than 15 years and resulted in nearly 90 joint publications, some of which I regard as my very best. It was a great partnership, and when I look back, I am amazed—on average, one publication every second month, including the years when Ulf qualified as a specialist in ophthalmology and entered a highly successful career in the clinic. We had complementary skills and worked very well together. Ulf was the imaginative experimentalist with a remarkable skill for advanced surgery in the rat brain, and I was the compulsive and driven thinker and the one organizing and leading the lab. An excellent combination, as it turned out!

In our discussions, Ulf came up with a somewhat odd but very clever idea: Why not challenge these axons with a noninnervated target and see whether these transected axons could regenerate a new noradrenergic innervation there? Ulf suggested that we use transplants of irises, placed so that they were in direct contact with the cut axons. Iris tissue was a classic experimental tool in Bengt's lab. This tissue has a rich adrenergic innervation, and it was in the trials using whole-mounts of irises that Bengt and Nils-Åke Hillarp obtained their first successful images of noradrenaline fluorescence in sympathetic nerves in 1961. Ulf reasoned that these thin tissue sheets—mostly composed of smooth muscle and nerves—would stand a good chance to receive sufficient nourishment through diffusion from the extracellular fluid to survive in the brain parenchyma. This proved to be right.

Ulf devised a technique using a thin, flat glass rod to introduce the iris through a hole in the skull, passing ventrally through the brain parenchyma and crossing the bundle carrying the axons from the locus coeruleus, the so-called dorsal noradrenergic bundle, as well as the dopamine and serotonin axons running within the medial forebrain bundle (MFB). When the rod was gently removed, the iris was left in place in direct contact with the cut stumps of these two fiber tracts. The results were striking: The regrowing catecholamine axons invaded the entire iris tissue within three to four weeks and remained densely innervated by six months after the surgery (Bjorklund and Stenevi 1971). We soon found out that the transplanted irises could be removed from the brain and unfolded so that it could be

spread out as a whole-mount on the microscope slide. Using this approach, we could study the dynamics and growth patterns of the regenerating noradrenaline axons in greater detail, all done in sympathectomised rats to exclude any involvement of sympathetic axons. In these whole-mounts, we could see that the regenerating noradrenergic axons growing from the transected dorsal bundle formed a well-developed terminal network. This network covered the entire iris within a month and mimicked very closely that of the normal sympathetic nerve supply, suggesting that the axons seek out and ramify along the denervated Schwann cell sheaths that form the so-called autonomic ground plexus within the smooth muscle of the iris (Svendgaard et al. 1975b). We went on to show that there was an extensive ingrowth of cholinergic axons as well, derived from the cholinergic axons running in the cingulum bundle and entering the transplants at their dorsal end. In double-stained whole-mounts, we could see that the noradrenergic and cholinergic innervations matched each other, suggesting that they ran together within the same neural sheets, as is the case in the intact iris (Svendgaard et al. 1976).

To us these findings provided compelling evidence that the same factors that promote and guide regeneration of peripheral sympathetic and parasympathetic axons can operate also on lesioned noradrenergic and cholinergic neurons in the brain and that this effect also was retained in iris transplants that were chronically denervated by at least a month before transplantation. The role of Schwann cells in axonal regeneration has been extensively studies over the years, and we know fairly well today that their role in promotion and guidance of regeneration peripheral axons is mediated by a mixture of growth factors, growth factor receptors, and cell-specific guidance molecules, some of which also are likely to operate in the CNS.

We published this extensive work in four papers in 1975–1976, one of them as a monograph in the Springer series called *Advances in Anatomy Embryology and Cell Biology* in 1975 (Svendgaard et al. 1975b). This turned out to be an unfortunate choice. According to Google Scholar this 76-page long paper has received zero citations over the ensuing years. What I am most proud of in this study are the illustrations. To be able to illustrate the complete outgrowth picture in the iris whole mounts, we used a combination of photomontages (composed of a grid of more than 50 individual pictures captured at 10 × magnification in the fluorescence microscope) and camera lucida-type ink drawings that I did in a large format. They look really nice and I still have one of these on the wall in my study.

Two other papers, published in *Nature* (Bjorklund et al. 1975b; Svendgaard et al. 1975a), received more attention (my first *Nature* papers!). In the first of these, we reported electrophysiological evidence of functional noradrenergic and cholinergic neurotransmission in the reinnervated transplants and concluded that

> an important implication of the current studies on the reinnervation of peripheral transplants in the CNS would be that—when given the same growth conditions as in the peripheral nervous system—at least some neurone systems in the mammalian CNS will show an entirely adequate regenerative capacity resulting in restitution of normal neurotransmission in the denervated tissue.

A few years later, this lead was picked up by Albert Aguayo in Montreal who went on to publish a series of seminal studies using grafts of peripheral nerves as conduits for regenerating axons in the spinal cord and the retinotectal system. One day in December 1979, he came on a visit to Lund to talk about his recent work on the regeneration of CNS neurons into nerve grafts, which was then in press in *Nature*. This was the beginning of a lifelong friendship that has involved not only stimulating scientific interactions and joint publications but also enjoyable contacts between our families.

Cell Transplantation: How It Started

In the neuroscience community, I am best known as one of the pioneers in the development of cell transplantation in the CNS. When I go back to the protocols in my picture archive, I see that Ulf and I made some early pilot experiments already in 1971 using neonatal sympathetic ganglia implanted directly into the brain parenchyma, but it was in the summer and autumn of 1973 that we took it up in a more serious way.

We were inspired not only by the intriguing results we had obtained in the experiments using transplants of iris and other peripheral tissues, but importantly also by Lars Olson's work at the Karolinska Institute in Stockholm. He had, as part of his PhD thesis, published a study on sympathetic ganglia transplanted to the anterior eye chamber in 1970, and in December 1972, Lars and his then-PhD student Åke Seiger published a paper in *Zeitschrift für Zellforschung* in which they reported observations from experiments using transplants of pieces of fetal and neonatal CNS tissue implanted into the anterior eye chamber. This paper caught my attention: Immature dopamine, noradrenaline, or serotonin neurons contained in small pieces of brain stem tissue, 1–3 mm^3, were shown to survive and grow after transplantation provided that they were taken from fetal donors. If they survive in the eye, it should be possible to make them survive also in the brain, I thought.

In the first series of experiments, we dissected pieces from the ventral midbrain (VM) and dorsal pons of late-stage fetal rat fetuses, similar in age to the ones used in the Olson and Seiger paper, and we deposited them into the VM of adult rats with the help of a metal cannula fitted with a plunger to extrude the tissue. Most of these transplants did not make it, but in some interesting cases, the dopamine and noradrenaline neurons, visualized with the F-H method, were seen to survive. After a while, we realized that these

transplants were mostly located in direct contact with the underlying pia, suggesting that this richly vascularized membrane could provide an important vascular support for the grafted tissue. This triggered Ulf to test an alternative approach, that is, to place the graft pieces directly onto the pia on the caudal surface of the midbrain. The approach he adopted was to suck away a piece of cortex, including a piece of the hippocampus, to expose the pia overlying the midbrain tectum. After all the bleeding was stopped (which could take some time), he placed the dissected piece on the pia and in direct contact with the cut surface of the hippocampus, and then filled the cavity with gelfoam. After some trial and error (apparent in the microscopy pictures I have saved in my photo archive), these efforts started to pay off. It was in the spring and summer of 1974 that we obtained the first results that convinced us that this was going to work. Although the outcome was still variable, the most successful specimens showed excellent survival of all three fetal neuron types, and also of pieces of superior cervical ganglia. The most striking finding, and the finding that made us really excited, was the extent of axonal outgrowth into the host hippocampus.

Over the next year, we worked to optimize and standardize the results. This concerned both the fetal donor age, the landmarks for dissection of the fetal tissue and refinement of the surgical procedure. We started to use rats treated with 6-OHDA or 5,7-DHT (to remove the noradrenergic and serotonergic innervation of the hippocampal formation), and Ulf introduced a second graft site: on the pia overlying the rostral thalamus, exposed through a suction lesion made to include the fimbria-fornix bundle. This lesion, which severed the connections between septum and the hippocampal formation, was effective in removing the cholinergic innervation of the entire hippocampal formation, save for its most ventral tip.

In the autumn of 1975, we were ready to publish the first two papers (Bjorklund et al. 1976; Stenevi et al. 1976). One contained a detailed account of the results with a focus on the rate of survival of fetal grafts placed in various locations, which we submitted to *Brain Research*, our household journal at the time. In the second paper, which we got published in *Nature,* we reported on the remarkable ability of grafted noradrenergic, dopaminergic, and serotonergic neurons to reinnervate the previously denervated hippocampus. A year later we followed this up with a study on the capacity of fetal septal cholinergic neurons to reinnervate the hippocampus in rats with lesions of the intrinsic septo-hippocampal pathway (Bjorklund and Stenevi 1977). The most intriguing finding in these studies was that different types of neurons, although growing along the same route, formed distinctly different and highly consistent terminal innervation patterns in the hippocampus. Thus, the innervation pattern generated by the grafted noradrenergic or cholinergic neurons appeared closely similar to that of the normal innervation patterns, while the terminal distribution of the dopaminergic and serotonergic innervations were entirely different. Moreover, the extent and patterning of

the ingrowing axons were entirely dependent on the presence or absence of the intrinsic afferent innervation of the same type.

Writing these papers gave me an opportunity to explore the earlier literature for previous attempts on neural transplantation in the mammalian brain. Neural tissue transplantation was of course a classic approach in cold-blooded vertebrates, but the attempts to explore this technique in mammals were more scattered and far between. I found that single reports, such as by Elisabeth Dunn (in 1917), Wilfried LeGros Clark (in 1940), and Gopal Das and Joe Altman (in 1971), showed that brain tissue from fetal or neonatal donors could survive, at least partly, in the brains of neonatal recipients. But our studies were apparently the first to achieve significant survival and growth of fetal CNS tissue in the brains of adult mammals. And we had a significant advantage over all previous studies: We had access to a microscopic technique, the F-H histofluorescence method, that allowed for visualization of specific subsets of neurons in their entirety. This made it possible for us to trace the outgrowing axons and map their terminal innervation fields in the host brain in a way that had not been possible before.

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. The regrowth of axons over long 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. 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. The innervation patterns of the intrinsic noradrenergic and cholinergic innervations (removed by lesions before transplantation) were accurately reproduced by the reinnervating axons derived from the grafted locus coeruleus and septal neurons, respectively. These exciting findings, which we published in 1976–1979, opened up a fascinating field of study on the functionality of the newly formed graft-host connections and their impact of hippocampus-related behaviors, and gave us new ideas of how cellular implants may be used to help the brain to repair itself.

Family

My marriage in 1976 had not been very long in the planning. I was known to work all the time, and one of my PhD students once asked me if I ever did anything else. My answer, as I recall it, was that I didn't need to do anything else.

Research was so fun, much like a dear hobby. I really enjoyed research, both the lab work and, in particular, the reading and writing that goes along with it, like a journey in a fascinating mental landscape, the world of the brain. I met Eva on New Year's Eve in 1974, in my hometown Växjö. This was at a party in the home of her aunt who at the same time was a close friend of my parents. She was, as I understood much later, just right for me, and I fell immediately in love.

Luckily, Eva lived also in Lund, and the next time we met was at the opera in Malmö where we saw a performance of the Strauss opera Der Rosenkavalier. It was a marvelous performance, as we both remember, one of many that would follow. This was like a seal, and since then, we have been together all the time. In May, we made a trip together to visit my brother's family in Stockholm, and on the travel back to Lund, I asked her if she wanted to join me on a trip to Canada, the United States, and Mexico in the autumn (the reason being that I had an invitation to give a talk at a meeting in Toronto, I must admit). Eva, as I found out later, does not like flying, so the thought of this trip terrified her. But luckily she said yes, and in October, we left on a two-week tour that took us first to Toronto, where I gave my talk and where we arranged to get engaged. Then off to San Diego (where I also gave a talk), and further on to Mexico City to see the pyramids and visit the remains of the Aztec culture (including a bout of Montezuma's revenge). When we were back in Lund, all went very fast. We found a house in the center of Lund that we could move into in February 1976, and in April, we got married in Eva's hometown with all our relatives present. Looking back, I realize that meeting Eva was the luckiest moment of my life. She has with a gentle hand steered my life and given me the balance between work and family that has made me prosper. Indeed, the decade after our two boys were born (in 1977 and 1979) was also the most creative and rewarding time in my research.

My Golden Decade

The long decade, from the publication of our first study of dopamine neuron transplants in the rat PD model in 1979 to the first report of dopamine neuron transplantation in PD patients, published in 1990 in *Science*, stands out as the most creative, dynamic, and enjoyable period in my scientific life. It included the beginning of lifelong friendships with two of my most stimulating collaborators and partners in science, Steve Dunnett and Rusty Gage, and the recruitment of some of my very best PhD students who have gone on to build highly successful careers on their own. And it was amazingly productive. Over this 12-year period, we published more than 100 original reports on studies using fetal cell transplantation in a broad range of experimental models, and we had 12 papers published in *Nature* or *Science*. On average one each year!

Of course, in those days, it was easier to get published in these journals—they were, like now, focused on novelty, but *Nature* and *Science* papers at the time typically reported observations from a single set of experiments, and they were often preliminary in nature meant to be followed by more substantial studies published in specialty journals. This was also the case for several of our *Nature* and *Science* papers. Our report of the mechanism of graft-induced functional recovery in the rat PD model, for example, published in *Nature* in 1981 (Bjorklund et al. 1981), was accompanied by more extensive and detailed studies in *Brain Research* published the same year (Dunnett et al. 1981a, 1981b). Similarly, our first study of the graft-induced recovery of maze learning in rats with lesions of the septo-hippocampal pathway published in *Nature* in 1982 (Low et al. 1982) was followed by several more extensive studies published in *Brain Research* in 1982 (Dunnett et al. 1982) and *Acta Physiologica Scandinavica* in 1983 (Bjorklund et al. 1983a, 1983b), and our first paper of neural grafting in the rat Huntington model, published in *Nature* in 1984 (Isacson et al. 1984) was followed by more extensive papers in *Neuroscience* and *PNAS* in 1985 and 1986 (Isacson et al. 1985, 1986).

Until 1979, I had run two very active research programs in parallel, one related to the anatomy of the catecholamine systems in the CNS, and one pursuing my interest in neuronal regeneration and intracerebral transplantation. Our pioneering cell transplantation studies, published in 1976–1979, had opened an entirely new field of investigation with unlimited possibilities. That really fascinated me. After 1979, most of our efforts were focused on the development of the cell transplantation approach.

I was lucky to have Steve and Rusty join me in this endeavor. Steve was a newly recruited doctoral student in Susan Iversen's lab in Cambridge, United Kingdom. I had met Sue at a meeting held at the Ciba Foundation (later Novartis Foundation) in London. She was one of the leading researchers in the study of the function of dopamine neurons in the brain, and in a break at the meeting, I asked her whether she would be interested in a collaboration to explore the functionality of intracerebral transplants. She was immediately positive and engaged Steve in this work. He 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 PD model, but also the functional effects of septal cholinergic neuron transplants in models of hippocampus-related memory and learning impairments.

In 1981, our work got a further boost when Rusty joined the lab. I met Rusty at the European Neuroscience meeting in Brighton in September 1980. We had a set of posters on our ongoing cell transplantation work. Rusty came to have a look, and we got engaged in interesting discussions. He had an interesting background in studies of hippocampus-related

behavior and was looking for a place to spend a sabbatical from his current job as assistant professor at Fort Worth in Texas. Initially, his plan was to stay for one year, but when that time was up, he decided to stay on for another three years in the lab. This was a fabulously stimulating and exciting time, simmering with new ideas! Rusty and Mary Lynn became dear friends, and when their daughters were born in Lund, it was as if we were all becoming a big extended family. In 1985, Rusty was recruited (by Bob Katzman!) to a faculty position at the University of California–San Diego (UCSD), and they moved back to the states. Luckily, we have managed to remain in close contact over the years.

In 1979, with Steve on board, we started to introduce new tools to monitor behavioral changes in rats with nigrostriatal or hippocampal lesions. Up until then, our work had been performed using microscopy techniques and biochemistry. Now when we had become seriously interested in studying recovery of function in our transplanted animals, we needed to be able to monitor behavioral changes in our lesioned rats. Urban Ungerstedt in Stockholm had recently introduced the rotation test to quantify loss of dopamine neurons in animals with unilateral 6-OHDA lesions of the nigrostriatal pathway, and he recently had constructed an automated "rotometer" for this purpose. Urban was kind to offer us to copy his equipment, and together with our engineer, Nils Lynhagen, I went to visit Urban's lab to see how that could be done. Following Urban's clever design, Nils prepared a bank of six rotometer bowls, and also automated open-field activity boxes that came to be extensively used in our lab over the following two decades. We bought our first desktop computers, two Swedish made ABC80 computers, running on BASIC and with floppy discs.

For our studies of the functional impact of grafted septal cholinergic neurons in rats with fimbria-fornix lesions, Steve had devised an elevated T-maze that we had built in our workshop. And a year later, in 1981, we were one of the first labs to adopt the Morris Watermaze test that had just been published. I had heard about it at the Society for Neuroscience meeting that was held in Los Angeles that year and thought that it would be a great tool for our studies of hippocampus-related behavior. We found a factory outside Lund that manufactured large plastic sewage tubes. Their largest diameter was 1.2 meters. I asked if they could sell us an 80-centimeter piece and attach it to a flat bottom. They were of course used to selling much longer ones—80 meter would have been more their style. But they kindly made it for us, and our caretaker brought it home tied to the roof of our department car. We found space for it in a temporary hut ("the blue barrack," bought from a local building company) that Bengt had put up next to our pink barrack. There we could establish our new behavior test lab and attach a sign on the door saying "World Brain Transplant Center."

With these new tools in hand, we could launch our studies on dopamine neuron transplants in the rat PD model (which I will deal with in more detail later) and studies on graft-induced recovery of maze learning in rats with lesions of the septo-hippocampal pathway, as well as in aged rats, which we pursued in parallel. Until then, Ulf and I had performed much of the experimental work ourselves, with the help of three highly skilled and experienced technicians, Gertrude Stridsberg, Birgit Haraldsson, and Ulla Jarl. Now as our transplantation work was getting up in speed, we started to look around for talented PhD students. In the autumn of 1980, we organized an evening "research-contact" course for interested medical students. Among the participants were two first-year students, Ole Isacson and Patrik Brundin. After the course, they contacted me to ask if they could follow the research in my lab. Yes, they were welcome to spend time in the lab, in parallel with their medical studies, and in the two following summers, they got going on experiments involving lesion and transplantation surgery in combination with behavioral tests.

In 1983, Ole and Patrik decided to take a break from their medical training and became enrolled as PhD students. In the beginning, they had worked together, which made it easier to mix the work with the scheduled activities in the medical classes, but now we had to define separate thesis projects. That was not easy, both of them were keen to work on the Parkinson project. There might be an opening, however: We had at that time switched from the solid-transplant/cavity method to transplantation using dissociated cell suspensions, and in a first round of experiments, we had tried this approach in rats with excitotoxin lesions of the striatum. This lesion had been introduced by Joe Coyle and Robert Schwarcz as a model of the striatal pathology seen in Huntington's disease, and our initial experiments, which were published in *Brian Research* in 1981 (Schmidt et al. 1981) had shown that the fetal striatal primordium, transplanted as a cell suspension, survived well in the excititoxically lesioned striatum and appeared to mature into a striatum-like structure. Thus, grafting in this alternative disease model could be an interesting topic! We agreed that these two projects were both interesting, and to solve who should do what, we drew lots: Patrik got the Parkinson project and Ole the Huntington one. A happy solution, and I think it worked out very well. Ole got his first *Nature* paper already in 1984, a follow-up *PNAS* paper in 1986, and defended his PhD thesis in 1987. Truly groundbreaking work that catapulted Ole to a highly successful career at Harvard in Boston. Patrik came to play a central role in the development of dopamine cell transplantation in patients with PD, as I will tell more about later. At the time when he defended his thesis, *Towards a Transplantation Therapy for Parkinson's Disease,* in May 1988, the first two patients had already been operated. The thesis defense was a memorable event. David Marsden, the leading movement disorders authority at the time, had been appointed as the faculty opponent. Patrik was off to a good start!

Cell Therapy for PD: How It Happened

Our first attempts to transplant fetal VM dopamine neurons to the striatum were performed in 1975. The approach we used was to prepare a suction cavity in the parietal cortex, through the corpus callosum, 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-splenial cavity, thus failing to provide the necessary vascular support. We reported these observations in our 1976 *Brain Research* paper 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 further experimentation, we found that we could make the fetal VM grafts survive by performing the surgery in two steps: In a first step, the cavity was prepared, filled with gel-foam, and closed. Three to four weeks later, the cavity was re-exposed and the fetal VM 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 of providing the underlying striatum with a new dense innervation that covered most of the dorsal caudate-putamen in the host.

We published these initial findings in 1979 (Bjorklund and Stenevi 1979). We submitted this paper first to *Science*, but while it was under review, a similar study, to our surprise, appeared in *Science*. 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 was based on the same idea, but their approach was different. In the *Science* paper, fetal VM tissue was implanted into the lateral ventricle, in contact with the surface of the denervated caudate-putamen. These transplants were quite small, and in contrast to the effective reinnervation seen in our transplanted animals, the growth of axons from the intraventricular transplants into the striatum was quite limited. We found that the functional impact of our intracavity grafts, monitored by tests of amphetamine-induced turning, was directly correlated to the extent of striatal reinnervation, 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 loss of dopamine innervation in the toxin-lesioned animals. The functional response induced by the intraventricular transplants, lacking the critical fiber outgrowth, was most probably due to dopamine diffusing from the grafted cells into the adjacent denervated striatum.

As a follow-up to the 1979 paper, and with Steve Dunnett on board, we sped up and published over the next two years 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 paper in *Nature* (Bjorklund et al. 1981), we proposed a model of dopamine graft function based on the idea that dopamine acts as a tonic regulatory system that sets the threshold for activation of the movement-initiating cortico-striato-thalamic circuitry. This suggested that the reinstatement of dopaminergic neurotransmission obtained by dopamine neuron transplants is able to reduce the threshold for the initiation of movements in response to movement-activating sensory stimuli. According to this model, tonic release of the transmitter at the reinnervated synaptic sites would be sufficient to induce recovery of sensori-motor behavior. In our subsequent studies of graft-induced dopamine release, which we studied using amperometry and intracerebral microdialysis, we could add further support for this idea. This mode of action helped to explain why dopamine neuron transplants placed in an ectopic location (i.e., in the striatum rather than in their normal location in the VM) are capable of restoring at least some aspects of normal dopamine-dependent behaviors. I believe it is still valid.

The cavity surgery used in these studies had an obvious weakness in that it involved major damage to the overlying cortex. And there was another snag: The functional effect seen in these animals was limited to recovery in motor asymmetry, and the severe and characteristic impairment in spontaneous sensori-motor behavior induced by the 6-OHDA lesion was not affected. Steve suggested that this shortcoming could be due to the fact that the new innervation extending from the intracortical grafts was limited to the dorsal part of the head of the caudate-putamen. In his ongoing thesis work, Steve had shown that the striatum is functionally heterogeneous and that it is the ventral-lateral sector, that is, the part corresponding to the putamen in humans, that is specifically involved in the regulation of sensori-motor behavior. Thus, more complete functional recovery may require more widespread reinnervation of the striatal complex.

We had already started to work on a different transplantation technique based on the injection of dissociated cell suspensions, which would allow grafting to deep brain sites, as well as multiple graft placements, and thus offer the possibility of obtaining more widespread and complete dopamine reinnervation of the striatum. Thus, in 1981, we abandoned the cavity approach in favor of a less invasive technique based on stereotaxic injections of dissociated cell suspensions directly into the brain parenchyma. The initial results we obtained with this approach were very promising, and in collaboration with Steve and Rusty, we set out to further optimize this technique and evaluate its potential, resulting in a series of experiments that was published (in six parts) as a supplement to *Acta Physiologica Scandinavica* in 1983 (Bjorklund et al. 1983c). The results showed that the cell suspension approach made it possible to distribute the graft tissue over multiple sites (up to five in these experiments) and achieve more extensive reinnervation of the striatum, combined with near-complete recovery of

both drug-induced and spontaneous sensori-motor behavior, as seen in rats with either unilateral or bilateral 6-OHDA lesions.

It was around this time that Olle and I initiated a process that would give us permission to use VM tissue from aborted human fetuses for transplantation. This allowed us to perform the necessary preclinical studies with human fetal tissue in our animal model, and it was Patrik who took this on. This preclinical work was summarized in three papers, published in 1986–1988, showing that human fetal VM tissue from seven- to nine-week-old fetuses could survive well after transplantation to the striatum (provided that the animals were immunosuppressed by daily injections of cyclosporine), reinnervate 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, Patrik was also able to identify the appropriate landmarks to be used for dissection of the dopamine-rich VM tissue pieces and define the optimal range of donor ages to be used in a future clinical protocol.

It was in 1985 that we started to discuss in earnest the possibility of initiating a clinical trial using VM tissue from aborted fetuses. But would it be possible to get the permission to use fetal tissue for this purpose? Even in Sweden with its liberal abortion law, this was a difficult and contentious issue. Professor David Ingvar, the head of clinical neurophysiology at our university, was at the time chairman of the Swedish Society of Medicine, and he advised us to engage the Society in a discussion of this issue. He put us in contact with the chairperson of its Ethics Delegation, and she agreed to take it on. After a series of hearings and discussions at the Society in Stockholm, guidelines for the use of human fetal tissue for transplantation purposes were adopted by the Society in March 1986 (later incorporated by the parliament into Swedish law). This was a critical step that made it possible for us to seek approval from our local ethical committees. But was the time ripe?

In April that year, Olle and I attended a meeting in New York, organized by the New York Academy of Sciences (NYAS). Olle reported on the results obtained in two patients who had received transplants of adrenal medullary tissue in the putamen (performed in 1985 in collaboration with the Karolinska team), and Patrik discussed the first results we had obtained with transplants of human fetal VM tissue in immunosuppressed rats. The discussions following these presentations were lively and raised a number of important issues. The ethical question, of course, was raised in the discussion after Patrik's talk, and he responded cautiously calling for public discussions on the use of human fetal tissue for transplantation to the brain. And then there was the question of the body of preclinical data needed to justify going into the clinic, and the push for studies in monkeys in particular. The 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) primate model had recently been introduced, and at the NYAS meeting, two teams, led by Roy Bakay and Eugene Redmond, had reported promising preliminary data in

MPTP-treated monkeys that had received transplants of fetal VM tissue into the caudate nucleus. Clearly, this model could offer an opportunity to validate the fetal grafting approach in a primate model. Nevertheless, Olle and I realized that it would take years to generate any really informative primate data, and we felt that in the end they would not tell us more than what we could learn from the rodent experiments. So when we returned home, we had decided to go ahead, in spite of the resistance from the scientific and clinical community.

Two months later, in June 1986, we were on our way to London to meet with David Marsden and Richard Frackowiak. Frackowiak's Medical Research Council (MRC) Cyclotron Unit at the Hammersmith Hospital has just adopted ^{18}F-DOPA as a tracer to monitor changes in dopamine metabolism using positron emission tomography (PET). This technique had been introduced a couple of years earlier by E. S. Garnett and collaborators, working at McMaster University in Canada, and the Hammersmith group had just published their first ^{18}F-DOPA study, not in PD, but in a patient with Huntington's disease. Garnett et al., in their 1984 paper, had shown that the accumulation of ^{18}F-DOPA was reduced in the striatum, and in the putamen in particular, in PD patients, and we realized that this technique could provide a tool to monitor survival of the transplanted dopamine neurons. But would this technique have sufficient resolution and sensitivity to detect a surviving graft? David and Richard were positive to give it a try, and after an intense year of preparations, including a series of "dry-run" rehearsals that we carried out in the summer and autumn of 1987, all was in place: the ethical permits, the well-rehearsed fetal dissection and handling procedure, the surgical approach, the assessment protocol, and the PET scanning protocol.

In September 1987, Olle traveled with the first two patients to Hammersmith for their preoperative ^{18}F-DOPA scans. This was the start of a long-lasting and very fruitful collaboration, initially driven by David, Richard, and Klaus Leenders, and later taken over by their successors Niall Quinn, David Brooks, and Paola Piccini. ^{18}F-DOPA PET became a central and important element of our program. This technique gave us the possibility to follow the development and survival of the grafted dopamine neurons over time, and with the increasing resolution of subsequent generations of PET scanners, it also allowed us to assess the extent of reinnervation provided by the grafts in individual patients. Later on, the Raclopride displacement method made it possible for us, for the first time, to monitor dopamine release from the grafted neurons. We often met the sour argument that it was the patient we should cure, not the PET scan. But these critics of course missed the point: Unless we could develop a procedure that allowed the grafted dopamine neurons to survive long term and restore dopamine neurotransmission back to normal, it would not be ready for more serious clinical testing. And there was also the issue of immune reactions and graft rejection. We knew from the animal experiments that allogeneic

transplants (i.e., transplants between unrelated individuals) survive well in the brain provided that the recipient is immunosuppressed. But we did not know what kind of immunosuppressive treatment would be necessary in the PD patients or how long it had to be maintained. The transplantation experts at the hospital advised us to use the same triple combination, cyclosporine, azathioprine, and prednisolone, they used for kidney transplants at the time. The access to ^{18}F-DOPA PET gave us the ideal tool to pick up signs of graft rejection, and with time, the PET scans have told us that temporary immunosuppression is sufficient to provide long-term graft survival over more than two decades.

The two first patients, operated in November and December 1987, were a disappointment. Both the clinical assessments and the ^{18}F-DOPA PET scans showed minimal improvement and no signs of graft survival. Olle presented the negative data at the International Parkinson Symposium in Jerusalem in 1988. We were disappointed, of course, but the reactions at the meeting were surprisingly positive and supportive. Olle was complimented for how carefully the study had been performed, and the amount of preclinical work that had gone into the design of the study was appreciated by many of our colleagues. So we returned home encouraged to proceed. First, however, we needed to make improvements in the transplantation procedure. We suspected that the transplantation instrument was too thick, with an outer diameter of 2.5 mm compared with the 0.45 mm thick syringe needle that we had used in the animal experiments. Together, with our neurosurgeon Stig Rehncrona, Patrik made a series of tests and showed that the excess damage and bleeding caused by the thicker cannula seriously compromised the survival of the cells. Indeed, when this first patient died in 2009, the autopsy confirmed that the graft had survived poorly (Kurowska et al. 2011). Clearly, we needed to use a thinner implantation device. Stig had one made by the hospital workshop, cleverly designed with an outer 1.0 mm cannula and an inner plunger that could be used both to load the tissue and to extrude it in defined steps.

With this new instrument in hand the two next patients, called Patient 3 and 4, were operated on in April and May 1989. They were transplanted unilaterally, on the side opposite to the most affected limbs, using VM tissue from four fetal donors. This time the number of implantation sites was increased from two to three to reach a larger volume of the putamen. Over the subsequent months, Olle had the patients come regularly for neurological assessment. In Patient 3, it was clear that something started to happen around three months: The speed of movements was increased, the rigidity in the limbs was gradually reduced, and the effect of his L-DOPA dose lasted longer. At five months, we decided that it was time for an ^{18}F-DOPA scan to check whether the improvement could be correlated with signs of a surviving graft. This was really exciting, and this time, I decided to join Olle in London to be there when it happened. Indeed, the scans showed a

clear increase in ^{18}F-DOPA uptake in the grafted putamen but not on the contralateral side. Subsequent scans, performed in both Patient 3 and 4, would show that the uptake in the grafted putamen continued to increase and reach normal levels after two or three years, in contrast to the uptake on the nongrafted side, which would continue to decrease over time.

To both Olle and me, this was one of the most exciting moments in our scientific careers. We rushed to publish, and when our paper appeared in *Science* in February 1990 (Lindvall et al. 1990), it caused an international media interest that none of us had anticipated. There were reports on our study, not only in Swedish media but also on CNN and BBC, and there were articles in the *New York Times* and *Washington Post*, and an editorial in *The Lancet* with the headline "A Brain Transplant That Works." We had reached an important milestone in our program.

Patient 4

Over the following decade, a total of 18 PD patients were transplanted in the Lund program. Olle has estimated that about one-third of them showed significant long-lasting and clinically meaningful improvements, accompanied by recovery of ^{18}F-DOPA uptake in the grafted putamen, and in some of the patients, the improvement was remarkable. One of them, Patient 4, stands out as quite special. He was transplanted one month after Patient 3 and remained with transplant on only one side. All other patients with unilateral transplants went on to receive a second transplant on the other side. This made him particularly interesting, since his nongrafted side could serve as an "internal control." He did very well after surgery and was able to withdraw his L-DOPA treatment completely after three years, and still at 10 years after transplantation, he had only mild Parkinsonian symptoms. At six years, Olle reintroduced a low dose of L-DOPA (one-third of the preoperative dose) owing to progression of symptoms on the side ipsilateral to the graft.

In 1998, the Hammersmith team published an interesting article in *Nature* in which they had introduced a new method to measure striatal dopamine release by PET. Paola Piccini suggested to Olle that this technique could be tried on the transplanted patients. Patient 4 was particularly interesting, because the measurements on the grafted side could be directly compared with those on the denervated, nongrafted side. The technique worked beautifully: The graft had indeed restored both spontaneous and drug-induced dopamine release (induced by a low dose of amphetamine) to normal levels in the grafted putamen. The release on the nonoperated side, by contrast, was reduced to only 10 percent of normal. The article reporting these findings, published in *Nature Neuroscience* in 1999 (Piccini et al. 1999), is undoubtedly one of the landmark papers in this field.

Patient 4 died in 2013, 24 years after transplantation. At that point, he had deteriorated and developed severe dementia. His brain was severely

atrophied, with enlarged ventricles, shrunken cortex, and Lewy body pathology distributed over all forebrain regions. Nevertheless, his transplant looked really great. In line with his 10-year PET scan, we found a dense, near-normal dopamine innervation in the grafted putamen, whereas the nongrafted putamen was completely devoid of dopamine fibers. To us, this was compelling proof that a rich functional dopamine innervation, which had developed over the first three years after transplantation and induced major and sustained symptomatic improvement, could be maintained for a quarter of a century in the PD brain. At the same time, the history of Patient 4 told us that the benefits of a viable transplant may be lost if widespread synucleinopathy and degenerative changes develop in the host brain. In the paper we published on these findings in *PNAS* (Li et al. 2016), I am particularly proud of the documentation of the microscopic material that Bengt Mattsson and I prepared from the TH-stained autopsy sections. Really beautiful, I think!

The Frozen Addicts

The discovery of MPTP is one of the most remarkable stories in the history of Parkinson's research. How this unraveled is like a detective story and has been very well told in the book *The Case of the Frozen Addicts* published in 1995 by Bill Langston and John Palfreman (new edition released in 2014). As told in this book, I first met Bill at a meeting in Belgium in October 1986, and under very memorable circumstances. We were on a bus taking us to a state dinner at Princess Liliane's Summer Palace outside Brussels. The rain was pouring down and, after some aimless driving in the misty darkness, it became clear to us that the driver had gotten lost. We had ended up in a narrow road that seemed to lead to nowhere, and when he tried to turn the bus around, it reversed into a field and got stuck in the mud. After some desperate attempts to get the bus loose, the driver decided to seek help. He locked the bus and walked away, and there we were, 18 scientists, all dressed up in tuxedos, left in the dark and listening to the rain pounding on the roof. I moved up to the front where there was some light and sat down beside Bill and we started to talk. I knew of course about Bill's famous MPTP patients, but this was the first time Bill heard about our cell transplantation program and our plans to try this in PD patients. He was intrigued and (as told in the book) found our plans fascinating. Indeed, it became immediately clear to us that the MPTP patients would be ideal candidates for this surgery: Their damage was likely to be confined to the dopamine system, their impairments were profound, but—unlike the human disease—stable and nonprogressive with little fluctuations, and the causative agent was no longer present. A very interesting idea!

So what happened to us in the bus? After about 40 minutes, a caravan of four private cars, with the desperate cook in the lead, came to pick us up.

We arrived an hour and a half late to a crowd of hungry people that had been kept going on sparkling wine.

Back in Lund, Olle liked the idea and agreed that the MPTP patients offered a very interesting opportunity. But it would require quite a bit of preparation to get everything in place. To work out the plans Olle and I went to visit Bill in California in July 1987. Bill took us on a tour of the sites where the MPTP drama had taken place, and he also arranged for us to meet his most severely affected patient, Connie Sainz, who was one of the candidates to be included. With Bill as a captivating guide, it became a highly memorable and absolutely fascinating experience. Then, in the beginning of December, Bill came to Lund to settle the plans: Håkan Widner, the younger of the two neurologists in the Lund team, would take the lead in this project, perform the presurgical assessments, select the two most suitable candidates to be included, and travel with the them to Lund for surgery.

Over the following year and a half, Håkan traveled frequently to Sunnyvale and also to Wisconsin, where one of the patients lived, to see the patients and perform the preclinical testing of those two, George and Juanita, who eventually were selected to take part in the trial. To cover the costs of Håkan's travels and hotel expenses, I had managed to get a donation from a small local family foundation run by the parents of a classmate of one of my children; they were surprised and perplexed when I contacted them, but I guess too embarrassed to say no! And Bill took an inventive step to cover the costs for the patients' travels to Sweden: The television program Nova wanted to make a follow-up of their previous documentary on the Frozen Addicts and John Palfreman had persuaded them to pay these costs provided that they were allowed to accompany the patients on their journey.

George was transplanted in August–September and Juanita in November 1989. They received transplants of VM tissue from four fetal donors, implanted on both sides with a two-week delay. The timing was good. We had just submitted our paper on Patient 3 to *Science,* giving us some confidence that we now had a grafting procedure that allowed the transplanted dopamine neurons to survive and function. The trial in the two MPTP patients, if positive, could provide further proof-of-principle for the technique we had developed.

The outcome was reassuring, but we had to wait for the positive results. During the first few months, very little happened. The first signs of improvement were observed at five to six months, and during the next two years, both of them showed a progressive improvement in motor function, accompanied by a marked increase in striatal ^{18}F-DOPA uptake that closely paralleled the clinical improvement in both patients. They could reduce their daily L-DOPA intake by 70 percent and the troublesome dyskinesias they had experienced before surgery were much reduced. They became much more independent, and the filmed sequences that appeared later in the

Nova program were striking. No question that the grafts had had a major impact on their Parkinsonian symptoms.

We published the results in November 1992 in *New England Journal of Medicine* (Widner et al. 1992). To our surprise, we found our paper back-to-back with two papers from two U.S. teams, reporting promising results obtained in idiopathic PD patients. Together these three studies caused a real upstir. In the United States, the use of tissue from aborted human fetuses was highly controversial and a ban on federal funding for fetal tissue research had been introduced by the Reagan administration already in 1988. The overall promising results reported in the three *NEJM* papers generated considerable interest and debate, and the resulting media attention may have played a role in the decision of the new president, Bill Clinton, to lift this ban in January 1993 (indeed, the first made in his presidency). This opened the way for the National Institutes of Health (NIH) to fund two sham surgery-controlled studies, the Colorado/Columbia trial and the Tampa/Mount Sinai/Rush trial, that were initiated in mid 1990s and reported in 2001 and 2003, respectively.

The negative outcome of these two trials was a major setback for the whole field and convinced many of our colleagues, particularly in the United States, that the cell transplantation approach had reached a dead-end. The wide media attention and the fact that these placebo-controlled trials were initiated and sponsored by the NIH helped to convey the impression that the outcome provided an authoritative and definitive answer: Cell transplantation in PD does not work. In retrospect, however, it is clear that these trials were performed prematurely. Cell transplantation was at the time in a very early stage of development and many technical issues and problems, such as cell preparation, handling and storage, method and site(s) of implantation, patient selection, and type and extent of immunosuppressive treatment, had not been studied properly and certainly not properly sorted out. Thus, the protocols used in the two trials were based on limited or insufficient experience from preclinical studies. Notably, these trials used limited immunosuppressive treatment (none in the Colorado/Columbia study) and the postoperative observation period was quite short, limited to one or two years. We learned later on that the clinical benefits induced by the transplants develop slowly over time, reaching full impact only after three to five years and that efficient immunosuppression is needed to obtain optimal graft survival. Clinical trials compromising on immunosuppressive treatment and using short end-points will thus run the risk of missing the full impact of the transplanted cells. Despite these shortcomings, the negative message spread by the NIH trials brought the whole field to a standstill. No further transplantations were made in Lund until last year when the European Union–sponsored TransEuro program was launched. The outcome of these new efforts will be known only in a few years' time.

Pathology Spread

In the early days, we were frequently asked whether the grafted neurons could be affected or damaged by the ongoing disease process. Little was known at that time about the pathophysiology of PD, so our response was, yes, this is definitely a possibility, but we do not know if this can happen. PD was known to be a progressive disease, but the mechanism underlying disease progression was not known. The impact of disease progression could go two ways: It might mask the effect of the graft, or it might attack the graft itself. Much later we would learn that both can occur, but that it takes a very long time.

The critical observation was made in 2007 when the brains from two of our transplanted patients had come to autopsy, 11 and 16 years after transplantation (Li et al. 2008). In these two brains Patrik and his senior collaborator Jia-yi Li observed that a small fraction of the TH-positive neurons in the grafts contained α-synuclein-positive aggregates, and some of them with the characteristic appearance of Lewy bodies, the pathological hallmark of PD. Similar changes were reported by Jeff Kordower in brains from the Tampa/Mount Sinai/Rush trial, and they seemed to be a consistent finding in all grafts that had survived longer than 10 years. The α-synuclein aggregates developed very slowly: About 2 percent of the TH neurons were affected at 11 years, 5 percent at 16 years and 11–12 percent at 24 years, suggesting that an additional 1 percent of the grafted neurons were recruited each year.

These findings generated enormous interest and opened up a completely new research field on the pathogenesis of PD. Our findings were viewed as the first compelling evidence for a prion-like mechanism underlying the spread of α-synuclein pathology that could explain its progression from the periphery to the brain and within the brain itself. This obviously represented a threat to the long-term viability of the graft, but it happened very slowly and involved only a small fraction of the cells. We knew that the fetal VM grafts could provide long-lasting and sustained benefit to the grafted patients and that the vast majority of the grafted dopamine neurons remained healthy and functional over more than a decade. To us, dopamine cell transplantation remained a viable therapeutic option.

Neurotrophic Factors: Nerve Growth Factor and Glial Cell Line-Derived Neurotrophic Factor

My collaboration with Rusty Gage also continued after his move to San Diego in 1985, but it was now focused on exploring the neuroprotective effects of nerve growth factor (NGF) in the septo-hippocampal system. In parallel with our studies on cholinergic neuron transplants in rats with lesions of the septo-hippocampal pathway, and in cognitively impaired aged

rats, Rusty and I had developed an interest in the basal forebrain cholinergic system, its reactions to injury, and its changes during aging. Work from Martin Schwab, Hans Thoenen, and Franz Hefti had provided interesting data showing that NGF was produced in the hippocampus and transported retrogradely in the septal cholinergic neurons and that exogenous NGF had a stimulation effect on ChAT activity in these neurons. This suggested that NGF may act as a neurotrophic factor, not only in the peripheral nervous system but also in the brain. Stimulated by these findings, we initiated a collaboration with Rusty's new colleague in San Diego, Silvio Varon, and his student Larry Williams, who had devised a method for continuous intraventricular infusion of NGF using osmotic minipumps. We applied this infusion technique in rats with unilateral lesions of the fimbria-fornix pathway, and the results were striking. The resulting paper, which we published in *PNAS* in 1986 (Williams et al. 1986), has become one of my most quoted publications. We went on to show that NGF-infused intraventricularly over four weeks could partially reverse the cholinergic neuron atrophy and improve retention of spatial memory seen in behaviorally impaired aged (two-year-old) rats (Fischer et al. 1987) and that similar infusions induced cholinergic neuron hypertrophy in rats before the onset of neuronal atrophy (Fischer et al. 1991).

Rusty went on to develop this approach further, and together with his colleagues at UCSD, Leon Thal and Mark Tuszynski, he confirmed this neuroprotective effect on lesioned septal cholinergic neurons in nonhuman primates and developed an entirely new approach to NGF delivery using transplants of fibroblasts engineered to secrete NGF. I was intrigued by this idea to combine cell transplantation and ex vivo gene transfer, and when Alberto Martinez-Serrano came to work as a postdoc in my lab, we decided to apply this technique to a conditionally immortalized neural progenitor cell line, HiB5, which we had obtained from Ron McKay in Boston. Alberto succeeded in generating a NGF-secreting HiB5 cell line that produced the factor at functionally active levels after transplantation to the brain, sufficient to afford complete rescue of axotomized cholinergic neurons after transplantation to the septum (Martinez-Serrano et al. 1995b) and equally effective at reversing age-dependent cognitive impairments and neuronal atrophy after transplantation to the basal forebrain in aged rats (Martinez-Serrano et al. 1995a).

It was at this time that Lin *et al.* reported the isolation and cloning of glial cell line-derived neurotrophic factor (GDNF). GDNF had been isolated as a potent neurotrophic factor for cultured dopamine neurons, and as soon as the paper was published in *Science* in May 1993, I started to explore the possibility of obtaining a sample to test in our new, progressive intrastriatal 6-OHDA lesion model. After several futile attempts, it was clear that Synergen (i.e., the company holding the ownership of GDNF) did not want to share the factor with us. So, instead, I turned to Arnon Rosenthal

at Genentech who was positive about helping us. In the meantime, the Synergen and Genentec teams had published two back-to-back papers in *Nature* providing evidence for a potent neurotrophic effect on lesioned dopamine neurons in mice and rats. In our study, which we published in *PNAS* in September 1995 (Sauer et al. 1995), we could show that injection of GDNF over the substantia nigra could provide complete dopaminergic (DA) neuron protection in the intrastriatal 6-OHDA model. The results were intriguing: GDNF was the first neuroprotective factor able to provide complete protection against neurotoxic damage in rodents as well as primates. We went on to characterize this effect in detail in rats, first using injections of recombinant protein over the nigra or into the striatum, and later using Lenti and adeno-associated virus (AAV) vectors to provide a source for constant delivery of GDNF. Over the following decade we published more than 20 papers (and material for four PhD theses) and established ourselves as one of the leading labs in the GDNF field.

It was this work that caught the attention of Teit Johansen, then working at the NeuroSearch company in Copenhagen. At a meeting in Copenhagen, where I had given a talk on our neurotrophic factor work, he contacted me and asked whether I would be interested in taking part in an initiative to establish a new gene therapy company devoted to the development of novel neuroprotective therapies. I agreed. This new company, NsGene, was launched in December 1999. This was the beginning of an enjoyable, and also quite exciting, endeavor that continued for more than a decade. We were lucky to have Lars Wahlberg join us as the third founding member. He was at the time working as research director of the CytoTherapeutics (CTI) company in Rhode Island, and when CTI closed down, Lars suggested that we should acquire the cell encapsulation technique from them. Over the next several years NsGene developed GDNF- and NGF-secreting cell lines to be used for encapsulation. These capsule devices were validated in both rats and minipigs, and manufactured to GMP standards. Because the company, despite serious efforts, failed to get a license for the use of GDNF, it was decided to develop the NGF-secreting capsules for a clinical trial in patients with Alzheimer's disease to be conducted in Stockholm (published in Eriksdotter-Jonhagen et al. 2012). Unfortunately, and despite the encouraging results obtained in this trial, the company failed to generate the financial support needed to continue these trials. But the company survived and under Lars' leadership, NsGene Inc. has now entered an agreement with Takeda to develop the encapsulation technology for PD.

The neurotrophic factor field has experienced its ups and downs, not unlike cell transplantation, and the efforts to use these factors clinically in patients with PD and Alzheimer's disease have encountered numerous setbacks. But we have not yet seen the end of this endeavor: GDNF in particular is endowed with such intriguing properties, not only because of its role as a survival factor but also because of its ability to promote neuronal

regeneration and boost function in lesion DA neurons. More recently, my research in this field has been focused on the interaction between Nurr1 and GDNF in the regulation of GDNF signaling, pointing to an interesting cellular machinery that appears to be dysregulated in nigral DA neurons affected by synuclein toxicity (Decressac et al. 2012).

The Wallenberg Center

In 1989–1990, I spent a 10-month sabbatical in Oxford. At that time, I felt it was time to take a break. I had had 20 years of intense research in Lund and felt that I needed to get away and contemplate on how to steer my research in the future. My two boys were now at the age, 10 and 12, when we thought they would benefit from the experience of living in another country and also training their English. I had an invitation from David Smith, the chairman of Pharmacology, to spend time in his MRC unit, and he offered to arrange a visiting professorship for me. This professorship was linked to Lincoln College and included housing in a superbly located villa next to the university park. A very nice offer and, luckily, Eva agreed to take leave of absence from her work at the museum in Malmö. It was a great experience for us all, and a very refreshing break for me. I had stimulating collaborations with Paul Bolam and Debbie Clarke that took some of my time, and my doctoral students came on visits for data analysis and writing of papers. Anyway, I had much time for reading and thinking, and all kinds of activities with my family.

Time was running fast, but all did not develop as I had expected. Soon after I had arrived, I was contacted by my close friends in Cambridge, asking if I was willing to join in a new initiative aimed to establish, and build, a new center for brain repair in Cambridge. They were in the process of submitting an application to the MRC and wanted to list me as the prospective director of this new center. It looked like a great plan and I agreed. Over the next few months, I drove to Cambridge for planning meetings, and I was summoned for a discussion with the MRC director, Dai Rees, about the realism of the plans. After several revisions, it was finally approved, but the awarded sum was only half of what had been requested, and most of it would be needed to cover the cost for the building. It became clear to me that the running costs would have to be generated from other sources. These prospects dampened my enthusiasm. In the meantime, however, I had become fond of the idea behind the project, a translational center on brain damage and repair spanning from molecules to man.

When I returned home to Lund by the end of May 1990 I contacted Per Belfrage, who at the time was vice-dean of the faculty (later dean) and asked whether there would be any possibility of establishing anything similar in Lund. The timing was good. Per had just started to dream up plans for a new research building—later to be named the Biomedical Center, or BMC—that would be a joint initiative between the university and the hospital and that

would allow the different departments of the medical faculty to be housed together. He convinced me that my idea could be realized as part of this plan, but if it was going to fly, it had to be a combined basic science–clinical initiative with a disease focus. I discussed this with Olle, and we agreed that it would be great to plan this as a joint venture. Per was very keen to get it off the ground. If we could secure financing of the Neuroscience Center, this could serve as a trigger for his greater BMC plan. The way to get it started was to attract a major donation from the Wallenberg Foundation, the largest research-funding agency in Sweden. But there was a snag: An application of this kind had to be submitted by the university, not by any constellation of scientists. Luckily, the vice-chancellor, Håkan Westling, was a close ally of Per and willing to help. Following informal contacts with the foundation, we were encouraged to prepare a proposal that could be incorporated into the formal application submitted by the vice-chancellor. We were lucky that the foundation awarded us a grant that would be sufficient to cover the cost for two floors in the new building, sufficient to house Olle's and my labs, with the rest to be covered by the university and the hospital. In the meantime, Per had secured support for the greater BMC plan from both the university and the hospital, and with the Wallenberg donation in hand, he could now take the next step, to initiate an architect competition. In the beginning of 1993, the plan was approved and the architects were chosen. Over the next year, we could work closely with Akademiska Hus and the architects in the detailed design of the labs in the Neuroscience Center. Once it got started, it all went very fast, and we could finally move into the new building in May 1996.

Entering a New Phase

The move to the new center was a major event, and an important milestone in my research. In the old histology building, we were cramped for space and the possibilities of modernizing and expanding the labs were limited. The new top-class facilities and the increased lab space in the center allowed me to expand my team and venture into new lines of research. As a result, my research became more diversified with several research programs developing in parallel and relying more on my senior postdocs, experienced technicians, and young colleagues in the day-to-day management of the work.

I also started to take my role as mentor more seriously. In the steadily increasing competition for positions and resources in academia, it has become increasingly difficult for young talented researchers to develop successful independent research careers. Here, senior established scientists with secure faculty positions can play an important role, not only as mentors but also in providing a genuinely supportive and well-equipped research environment that can help our best young investigators get started and establish independent research programs, and to be able to compete successfully for major grants and well-financed group leader positions. It has been one

of the most rewarding experiences in my research life to see many of my best PhD students and postdocs go on to develop highly successful research careers, both in Lund and in other universities around the world. As a scientist, I am blessed with a large "extended family."

The 1990s was a very busy time for me in many other ways. I had been elected to the Swedish Academy of Sciences in 1989 and came to serve as the chairman for the medical section for five years, 1995–2000. I had also become very much involved with the European Neuroscience Association (ENA), first as a council member and later as president. I was elected to the ENA Council in 1985, and over the subsequent 11 years, I took part in two major initiatives, the launch of the *European Journal of Neuroscience* and the creation of the Federation of European Neuroscience Societies (FENS). Inspired by the example of SfN's *Journal of Neuroscience*, ENA decided in the late 1980s to start its own journal to generate income for the association. They appointed four of us, Per Andersen, Michel Cuenod, Wolf Singer, and myself ("the gang of four") to get it done. We managed to find a publisher (Oxford University Press) and were very lucky to persuade Ray Guillery to take on the job as the journal's first chief editor. It became a success. The revenues were relatively modest during the first few years, but with time, it has become economically profitable and has given the society a solid economic basis to grow and become the leading force in European neuroscience.

It was during my time as president elect and president, 1994–1998, that ENA was transformed into the Federation of European Neuroscience Societies. In the beginning of the 1990s, the attendance of the annual ENA meetings had declined, to less than 2,000, an order of magnitude below the American SfN meetings, and there was a worry that more and more of the European scientists had chosen to go to the ever-growing SfN meetings instead. The problems facing the ENA meetings were distinctly European: the competition from large national neuroscience meetings held during the same years, and the presence of around 20 specialized European associations attracting participants from a range of subdiciplines. It was decided to replace ENA with a federation of national societies that would hold a joint European meeting every two years, linked to a deal with the national societies promising to hold their meetings in the intervening years. At first, there was resistance from the well-established national societies, and there was in several cases a reluctance to change to a bi-annual meeting schedule. But in the end, we managed to get all major societies on board and the first Federation meeting, called Forum of European Neuroscience, was held in Berlin in 1998. The organization of the Berlin meeting, which was in the hands of Helmut Kettenmann and his team, was a financial gamble: Despite a highly cost-effective organization, the meeting had to have more than 3,000 paying registrants to break even, twice as many as the previous ENA meetings. In the end, there were more than 4,000 registrants, and to house

the increased numbers of posters, we managed to get *Nature Neuroscience,* which had just launched their first issues, to sponsor a large tent that was erected outside the meeting room. Since then, the FENS Forum meetings have continued to grow to become a truly international meeting, attended by more than 6,000 delegates from around the world.

Gene Therapy and Viral Vectors

After his move to San Diego, Rusty teamed up with one of the pioneers in the gene therapy field, Ted Friedman, and initiated a new line of research based on the transplantation of cells engineered to secrete therapeutic factors. They developed NGF-secreting fibroblasts and transplanted them to rats with fimbria-fornix lesions, (i.e., the same model we had used in our NGF infusions experiments). In a paper published in *Science* in December 1988, they could show similar protective and growth-stimulating effects on the axotomized cholinergic neurons as we had obtained with protein infusions. Soon thereafter, in September 1989, Jacques Mallet in Paris published a paper in *PNAS* showing that cells could be engineered to stably produce L-DOPA and dopamine by the use of retroviral gene transfer.

This approach intrigued me. Here was a new possibility for cell-based dopamine replacement, and I was keen to explore this idea further. I met Jacques at a meeting in Paris in October and suggested that we should collaborate. I checked with Patrik, and he was positive to join as well. We moved quickly, and with Patrik's help, we transplanted two different cell lines, one secreting only DOPA and one secreting mainly dopamine. Both cell types survived implantation into the dopamine-depleted striatum and expressed TH immunoreactivity. Using the microdialysis technique, we could show that the DOPA-producing cells secreted large amounts of DOPA that was efficiently decarboxylated to dopamine by the host striatal tissue. They produced four to five times higher extracellular dopamine levels than the dopamine-secreting cells, and they were also functionally more efficient. We submitted the paper to *Neuron* in May and it was out in print in October (Horellou et al. 1990). Not bad—from idea to a high-impact publication in just over a year!

The European Science Foundation had just launched a new program for funding transnational projects in neuroscience. Together with Jacques and his team, we submitted a joint proposal and got funded. This grant made it possible for us to develop this new gene therapy approach, and it provided the money to recruit a new PhD student to this project, Cilla Lundberg. She became the driving force in these projects, and after her thesis defense in 1996, she was the one who led the transition from engineered cells to the use of viral vectors, a technology that has been central to the work in our center ever since.

The viral vector technology led to the development of two major research programs. The first program involved the use of AAV vectors expressing TH and GCH1 for local delivery of L-DOPA. This new line of work started when Ron Mandel spent his second postdoc period in my lab in 1999. He was between jobs, moving from his previous job at the Somatix company to his new job in Gainesville, Florida, and had been allowed by Somatix to use his TH- and GCH1-expressing vectors for experiments here in Lund. This study, which was published in *PNAS* in 2002 (Kirik et al. 2002a), was the beginning of a program that eventually led us to start our company, *Genepod Therapeutics*, with the goal to bring this gene therapy approach to the clinic. The second program investigated the use of AAV vectors to overexpress α-synuclein in nigral dopamine neurons as a way to reproduce the synuclein toxicity seen in patients with PD. The role of α-synuclein in PD had been discovered a few years earlier, and the generation of rodent models of what was coined "synucleiopathy" was a hot topic. We were among the first to develop viral vector models of PD that reproduced several of the cardinal features of the human disease. Our first two papers, published in 2002 (in rats; Kirik et al. 2002b) and 2003 (in monkeys; Kirik et al. 2003), have become standard references in this field. Work based on this model, used as a tool to study the pathogenesis of PD and as an experimental model for neuroprotective and disease-modifying treatments, has been central to the work in my lab up to the present day.

Stem Cells: My Road to the Future

My interest in expandable neural progenitors goes back to the early 1990s when techniques for expansion of neural progenitors in the presence of growth factors were developed by Brent Reynolds and Sam Weiss, who developed the neurosphere culture system, and by Rusty and his collaborators, who worked with adherent monolayer cultures. I was intrigued by these new techniques. The use of multipotent progenitors that could be expanded to large numbers in culture suggested a possibility of bypassing the use of fetal cells for grafting. We adopted the neurosphere technique in my lab, and through a collaboration with Melissa Carpenter and Lars Wahlberg at the CytoTherapeutics company, we got access to a stable human neurosphere cell line that we could explore in our transplantation models. We transplanted these cells into the neurogenic regions in the adult rat brain—the subventricular zone, and the dentate gyrus—and showed for the first time that these in vitro propagated human progenitors could respond to in vivo guidance cues, allowing them to migrate specifically along the routes normally taken by the endogenous neuronal precursors: along the rostral migratory stream to the olfactory bulb and within the subgranular zone in the dentate gyrus, with site-specific neuronal differentiation in the granular and periglomerular layers of the bulb and in the dentate granular cell layer (Fricker et al. 1999).

During this time, we worked hard to generate dopamine neurons from neurosphere progenitors, but eventually we gave up: the neurospheres were too heterogeneous in composition, and in our hands, they were largely biased to develop into glial phenotypes in the brain. The important breakthrough in this research had to wait until my former student and postdoc, Malin Parmar, started to work with human embryonic stem cells. After her PhD in 2003, she went to work on embryonic stem cells with Meng Li in Edinburgh, and brought this knowledge back with her to Lund. Eventually, and in close collaboration with a new recruit, Agnete Kirkeby, she managed to devise a protocol for efficient conversion of human embryonic stem cells to dopamine neurons that possess the properties of authentic midbrain dopamine neurons. The paper reporting the performance of these cells in vivo, after transplantation to the striatum and substantia nigra in the 6-OHDA lesion model, published in *Cell Stem Cell* in 2014 (Grealish et al. 2014) is an important landmark in our efforts to generate transplantable dopamine neurons from stem cells, and a critical step toward their clinical use in PD patients.

Epilogue

Our field has in the past two decades seen two major breakthroughs: the discovery of human embryonic stem cells by Jamie Thompson and collaborators in 1998, and the introduction of cellular reprogramming by Shinya Yamanaka in 2006. These technical innovations have opened up entirely new possibilities and generated important new tools for cell- and gene-based brain repair. The introduction of these new techniques has been truly transformative and has given new momentum to our field. As a result, the research closest to my heart—the development of cell-replacement for brain repair—has over the past decade entered a new exciting phase and made it possible for our lab at the Wallenberg Center, now led by Malin Parmar and her team, to join the frontline in the development of a stem cell–based therapy for PD. Together with our partners in the international consortium, called *G-Force-PD,* which was established four years ago, we are now part of a coordinated international effort to bring this technology to be tested in the clinic. As I write this, in September 2017, it is 40 years since Ulf and I made our first exploits in cell transplantation and almost exactly 30 years since we operated on our first PD patient. Looking back, I feel both lucky and proud to be part of this exciting new endeavor that builds on the program we started and developed here in Lund. In many ways, it is a continuation of what Olle and I dreamed up 30 years ago.

References

Baumgarten HG, Bjorklund A, Lachenmayer L, Nobin A. 1973. Evaluation of the effects of 5,7-dihydroxytryptamine on serotonin and catecholamine neurons in the rat CNS. *Acta Physiol Scand Suppl* 391: 1–19.

Baumgarten HG, Bjorklund A, Lachenmayer L, Nobin A, Stenevi U. 1971. Long-lasting selective depletion of brain serotonin by 5,6-dihydroxytryptamine. *Acta Physiol Scand Suppl* 373: 1–15.

Bjorklund A, Baumgarten HG, Rensch A. 1975a. 5,7-Dihydroxytryptamine: improvement of its selectivity for serotonin neurons in the CNS by pretreatment with desipramine. *J Neurochem* 24: 833–5.

Bjorklund A, Enemar A, Falck B. 1968. Monoamines in the hypothalamo-hypophyseal system of the mouse with special reference to the ontogenetic aspects. *Z Zellforsch Mikrosk Anat* 89: 590–607.

Bjorklund A, Falck B, Hromek F, Owman C, West KA. 1970. Identification and terminal distribution of the tubero-hypophyseal monoamine fibre systems in the rat by means of stereotaxic and microspectrofluorimetric techniques. *Brain Res* 17: 1–23.

Bjorklund A, Falck B, Rosengren E. 1967. Monoamines in the pituitary gland of the pig. *Life Sci* 6: 2103–10.

Bjorklund A, Gage FH, Schmidt RH, Stenevi U, Dunnett SB. 1983a. Intracerebral grafting of neuronal cell suspensions. VII. Recovery of choline acetyltransferase activity and acetylcholine synthesis in the denervated hippocampus reinnervated by septal suspension implants. *Acta Physiol Scand Suppl* 522: 59–66.

Bjorklund A, Gage FH, Schmidt RH, Stenevi U, Dunnett SB. 1983b. Intracerebral grafting of neuronal cell suspensions. VII. Recovery of choline acetyltransferase activity and acetylcholine synthesis in the denervated hippocampus reinnervated by septal suspension implants. *Acta Physiol Scand Suppl* 522: 59–66.

Bjorklund A, Johansson B, Stenevi U, Svendgaard NA. 1975b. Re-establishment of functional connections by regenerating central adrenergic and cholinergic axons. *Nature* 253: 446–8.

Bjorklund A, Nobin A, Stenevi U. 1973. Regeneration of central serotonin neurons after axonal degeneration induced by 5,6-dihydroxytryptamine. *Brain Res* 50: 214–20.

Bjorklund A, Stenevi U. 1971. Growth of central catecholamine neurones into smooth muscle grafts in the rat mesencephalon. *Brain Res* 31: 1–20.

Bjorklund A, Stenevi U. 1977. Reformation of the severed septohippocampal cholinergic pathway in the adult rat by transplanted septal neurons. *Cell Tissue Res* 185: 289–302.

Bjorklund A, Stenevi U. 1979. Reconstruction of the nigrostriatal dopamine pathway by intracerebral nigral transplants. *Brain Res* 177: 555–60.

Bjorklund A, Stenevi U, Dunnett SB, Iversen SD. 1981. Functional reactivation of the deafferented neostriatum by nigral transplants. *Nature* 289: 497–9.

Bjorklund A, Stenevi U, Schmidt RH, Dunnett SB, Gage FH. 1983c. Intracerebral grafting of neuronal cell suspensions. I. Introduction and general methods of preparation. *Acta Physiol Scand Suppl* 522: 1–7.

Bjorklund A, Stenevi U, Svendgaard N. 1976. Growth of transplanted monoaminergic neurones into the adult hippocampus along the perforant path. *Nature* 262: 787–90.

Bjorklund A, Wiklund L. 1980. Mechanisms of regrowth of the bulbospinal serotonin system following 5,6-dihydroxytryptamine induced axotomy. I. Biochemical correlates. *Brain Res* 191: 109–27.

Decressac M, Kadkhodaei B, Mattsson B, Laguna A, Perlmann T, Bjorklund A. 2012. alpha-Synuclein-induced down-regulation of Nurr1 disrupts GDNF signaling in nigral dopamine neurons. *Sci Transl Med* 4: 163ra56.

Dunnett SB, Bjorklund A, Stenevi U, Iversen SD. 1981a. Behavioural recovery following transplantation of substantia nigra in rats subjected to 6-OHDA lesions of the nigrostriatal pathway. I. Unilateral lesions. *Brain Res* 215: 147–61.

Dunnett SB, Bjorklund A, Stenevi U, Iversen SD. 1981b. Behavioural recovery following transplantation of substantia nigra in rats subjected to 6-OHDA lesions of the nigrostriatal pathway. II. Bilateral lesions. *Brain Res* 229: 457–70.

Dunnett SB, Low WC, Iversen SD, Stenevi U, Bjorklund A. 1982. Septal transplants restore maze learning in rats with fornix-fimbria lesions. *Brain Res* 251: 335–48.

Eriksdotter-Jonhagen M, Linderoth B, Lind G, Aladellie L, Almkvist O, et al. 2012. Encapsulated cell biodelivery of nerve growth factor to the Basal forebrain in patients with Alzheimer's disease. *Dement Geriatr Cogn Disord* 33: 18–28.

Fischer W, Bjorklund A, Chen K, Gage FH. 1991. NGF improves spatial memory in aged rodents as a function of age. *J Neurosci* 11: 1889–906.

Fischer W, Wictorin K, Bjorklund A, Williams LR, Varon S, Gage FH. 1987. Amelioration of cholinergic neuron atrophy and spatial memory impairment in aged rats by nerve growth factor. *Nature* 329: 65–8.

Fricker RA, Carpenter MK, Winkler C, Greco C, Gates MA, Bjorklund A. 1999. Site-specific migration and neuronal differentiation of human neural progenitor cells after transplantation in the adult rat brain. *J Neurosci* 19: 5990–6005.

Grealish S, Diguet E, Kirkeby A, Mattsson B, Heuer A, et al. 2014. Human ESC-derived dopamine neurons show similar preclinical efficacy and potency to fetal neurons when grafted in a rat model of Parkinson's disease. *Cell Stem Cell* 15: 653–65.

Horellou P, Brundin P, Kalen P, Mallet J, Bjorklund A. 1990. In vivo release of dopa and dopamine from genetically engineered cells grafted to the denervated rat striatum. *Neuron* 5: 393–402.

Hornykiewicz, O. *The History of Neuroscience in Autobiography*, Vol. 4, 2004, pp 242–281.

Isacson O, Brundin P, Gage FH, Bjorklund A. 1985. Neural grafting in a rat model of Huntington's disease: progressive neurochemical changes after neostriatal ibotenate lesions and striatal tissue grafting. *Neuroscience* 16: 799–817.

Isacson O, Brundin P, Kelly PA, Gage FH, Bjorklund A. 1984. Functional neuronal replacement by grafted striatal neurones in the ibotenic acid-lesioned rat striatum. *Nature* 311: 458–60.

Isacson O, Dunnett SB, Bjorklund A. 1986. Graft-induced behavioral recovery in an animal model of Huntington disease. *Proc Natl Acad Sci USA* 83: 2728–32.

Katzman R, Bjorklund A, Owman C, Stenevi U, West KA. 1971. Evidence for regenerative axon sprouting of central catecholamine neurons in the rat mesencephalon following electrolytic lesions. *Brain Res* 25: 579–96.

Kirik D, Annett LE, Burger C, Muzyczka N, Mandel RJ, Bjorklund A. 2003. Nigrostriatal alpha-synucleinopathy induced by viral vector-mediated overexpression of human alpha-synuclein: a new primate model of Parkinson's disease. *Proc Natl Acad Sci USA* 100: 2884–9.

Kirik D, Georgievska B, Burger C, Winkler C, Muzyczka N, et al. 2002a. Reversal of motor impairments in parkinsonian rats by continuous intrastriatal delivery of L-dopa using rAAV-mediated gene transfer. *Proc Natl Acad Sci USA* 99: 4708–13.

Kirik D, Rosenblad C, Burger C, Lundberg C, Johansen TE, et al. 2002b. Parkinson-like neurodegeneration induced by targeted overexpression of alpha-synuclein in the nigrostriatal system. *J Neurosci* 22: 2780–91.

Kurowska Z, Englund E, Widner H, Lindvall O, Li JY, Brundin P. 2011. Signs of degeneration in 12-22-year old grafts of mesencephalic dopamine neurons in patients with Parkinson's disease. *J Parkinsons Dis* 1: 83–92.

Li JY, Englund E, Holton JL, Soulet D, Hagell P, et al. 2008. Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. *Nat Med* 14: 501–3.

Li W, Englund E, Widner H, Mattsson B, van Westen D, et al. 2016. Extensive graft-derived dopaminergic innervation is maintained 24 years after transplantation in the degenerating parkinsonian brain. *Proc Natl Acad Sci USA* 113: 6544–9.

Lindvall O, Bjorklund A. 1974. The organization of the ascending catecholamine neuron systems in the rat brain as revealed by the glyoxylic acid fluorescence method. *Acta Physiol Scand Suppl* 412: 1–48.

Lindvall O, Bjorklund A, Hokfelt T, Ljungdahl A. 1973. Application of the glyoxylic acid method to vibratome sections for the improved visualization of central catecholamine neurons. *Histochemie* 35: 31–8.

Lindvall O, Bjorklund A, Skagerberg G. 1984. Selective histochemical demonstration of dopamine terminal systems in rat di- and telencephalon: new evidence for dopaminergic innervation of hypothalamic neurosecretory nuclei. *Brain Res* 306: 19–30.

Lindvall O, Brundin P, Widner H, Rehncrona S, Gustavii B, et al. 1990. Grafts of fetal dopamine neurons survive and improve motor function in Parkinson's disease. *Science* 247: 574–7.

Loren I, Bjorklund A, Falck B, Lindvall O. 1980. The aluminum-formaldehyde (ALFA) histofluorescence method for improved visualization of catecholamines and indoleamines. I. A detailed account of the methodology for central nervous tissue using paraffin, cryostat or Vibratome sections. *J Neurosci Methods* 2: 277–300.

Low WC, Lewis PR, Bunch ST, Dunnett SB, Thomas SR, et al. 1982. Function recovery following neural transplantation of embryonic septal nuclei in adult rats with septohippocampal lesions. *Nature* 300: 260–2.

Martinez-Serrano A, Fischer W, Bjorklund A. 1995a. Reversal of age-dependent cognitive impairments and cholinergic neuron atrophy by NGF-secreting neural progenitors grafted to the basal forebrain. *Neuron* 15: 473–84.

Martinez-Serrano A, Lundberg C, Horellou P, Fischer W, Bentlage C, et al. 1995b. CNS-derived neural progenitor cells for gene transfer of nerve growth factor to the adult rat brain: complete rescue of axotomized cholinergic neurons after transplantation into the septum. *J Neurosci* 15: 5668–80.

Piccini P, Brooks DJ, Bjorklund A, Gunn RN, Grasby PM, et al. 1999. Dopamine release from nigral transplants visualized in vivo in a Parkinson's patient. *Nat Neurosci* 2: 1137–40.

Sauer H, Rosenblad C, Bjorklund A. 1995. Glial cell line-derived neurotrophic factor but not transforming growth factor beta 3 prevents delayed degeneration of nigral dopaminergic neurons following striatal 6-hydroxydopamine lesion. *Proc Natl Acad Sci USA* 92: 8935–9.

Schmidt RH, Bjorklund A, Stenevi U. 1981. Intracerebral grafting of dissociated CNS tissue suspensions: a new approach for neuronal transplantation to deep brain sites. *Brain Res* 218: 347–56.

Skagerberg G, Bjorklund A, Lindvall O, Schmidt RH. 1982. Origin and termination of the diencephalo-spinal dopamine system in the rat. *Brain Res* Bull 9: 237–44.

Skagerberg G, Lindvall O, Bjorklund A. 1984. Origin, course and termination of the mesohabenular dopamine pathway in the rat. *Brain Res* 307: 99–108.

Stenevi U, Bjorklund A, Svendgaard NA. 1976. Transplantation of central and peripheral monoamine neurons to the adult rat brain: techniques and conditions for survival. *Brain Res* 114: 1–20.

Svendgaard NA, Bjorklund A, Hardebo JE, Stenevi U. 1975a. Axonal degeneration associated with a defective blood-brain barrier in cerebral implants. *Nature* 255: 334–6.

Svendgaard NA, Bjorklund A, Stenevi U. 1975b. Regenerative properties of central monoamine neurons: studies in the adult rat using cerebral iris implants as targets. *Adv Anat Embryol Cell Biol* 51: 3–77.

Svendgaard NA, Bjorklund A, Stenevi U. 1976. Regeneration of central cholinergic neurones in the adult rat brain. *Brain Res* 102: 1–22.

Widner H, Tetrud J, Rehncrona S, Snow B, Brundin P, et al. 1992. Bilateral fetal mesencephalic grafting in two patients with parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *N Engl J Med* 327: 1556–63.

Wiklund L, Bjorklund A. 1980. Mechanisms of regrowth in the bulbospinal serotonin system following 5,6-dihydroxytryptamine induced axotomy. II. Fluorescence histochemical observations. *Brain Res* 191: 109–27.

Williams LR, Varon S, Peterson GM, Wictorin K, Fischer W, et al. 1986. Continuous infusion of nerve growth factor prevents basal forebrain neuronal death after fimbria fornix transection. *Proc Natl Acad Sci USA* 83: 9231–5.