

Restorative Therapies for Parkinson's Disease: Ethical Issues

GERARD J. BOER

1. Introduction

Improved health care and the many therapies and surgery methods for all kinds of previously life-threatening diseases have helped to increase the average lifespan of the world population. As a consequence, the incidence of disease connected with old age, in particular degenerative diseases of the brain, has increased: the human body outlives the normally aging brain. One of these diseases is Parkinson's disease (PD), in which the gradual loss of the neurons of the substantia nigra serving the dopaminergic input of the brain caudate/putamen complex seems the key cause of the prominent movement dysfunctions typically related to the disease. There is a relatively satisfactory pharmacological treatment for the early disease symptoms, which is based on promoting dopamine production to therapeutic levels in those nigra cells that are still present. The treatment is, however, not ideal. It fails in later stages of the disease when nigra cell loss has progressed, because it does not stop the neurodegeneration of the nigro-striatal system. For several decades now, PD has been subject of intense fundamental research aimed at bringing about repair of the brain in this large group of patients. In particular, the prospect of brain restorative therapies, such as cell or gene therapy, has raised enormous interest and is seen as a new method to tackle the disease more efficaciously. The ethical issues involved, both from the methodological angle as well as from the patient's point of view, involved in the development of such treatments are subject of the present chapter.

Department of Neuroregeneration, Netherlands Institute for Neurosciences, Amsterdam, The Netherlands
g.boer@nin.knaw.nl

2. Variety of New Treatment Approaches

Classic therapies for PD are all designed to restore striatal dopamine signaling, using oral preparations of the precursor L-DOPA, dopamine agonists, or drugs synthesized to enhance the efficacy of endogenous dopamine by inhibition of dopamine breakdown. In the early disease stages these therapies are effective, so brain restorative interventions are of more importance in the later stages of the disease when drug treatment starts to falter and major complications develop (Calne, 1993; Ahlskog and Muentert, 2001). Therapies like deep brain stimulation and lesioning (Benabid, 2003) are used to treat PD patients in cases extreme motor handicaps, but repair of the brain by cell or gene therapeutic surgery without lifelong or long-lasting post-operative care remains the ultimate goal. If successful, these therapies might even be considered in the early stages of PD in order to prevent the side effects of the current drug treatments.

Cellular therapies are meant either to replace lost nigra neurons or to supply cells that can compensate for the loss of dopamine production (cellular vs. molecular replacement) (Björklund et al., 2003). Molecular replacement may also be achieved by encapsulated dopamine-producing cell preparations, or by in vivo gene transfer for the enzyme system necessary for the dopamine production. Cell and gene therapy recently also became of importance for regenerative neurosurgery in different ways: namely, the introduction of neurotrophic factors, that may stop degeneration or direct regeneration, or of enzymes for GABA production that can counteract the imbalance of the striatal output pathways as achieved by electric hyperstimulation or GABA agonist infusion in the subthalamic nucleus (STN) in PD patients. Thus, restorative surgery in PD is developing in many directions.

Autologous neuroimplantation of adrenal medulla tissue, potentially producing dopamine, was the first neurorestorative clinical trial in PD (Bäcklund et al., 1985). Autologous peripheral dopaminergic neuron implants are yet another means of substituting for the lost dopamine in the striatum (Itakura et al., 1997). However, allotransplantation of fetal mesencephalic primary dopaminergic neurons became the new approach that was rather widely studied in patients. It can significantly suppress the disease symptoms in the later stages of PD, despite serious side effects (Dunnett et al., 2001). Further momentum in cell therapeutic approaches in PD was supplied by the potential of embryonic (ES) and somatic stem (SS) cells as source cells for neurotransplantation (Armstrong and Svendsen, 2000; Brazelton et al., 2000; Mezey et al., 2000; Donovan and Gearhart, 2001; Toma et al., 2001). Using human embryos and fetuses as sources of cell or tissue implants in the brain of patients with neurological or psychiatric disorders has not been and is still not acceptable for parts of society. Ethical considerations regarding the retrieval and use of this donor material have led to the drawing up of guidelines. The recent isolation of human ES (hES) cells, in order to grow and differentiate these cells for organ repair, has given new impetus

to societal and political debates on this issue (McLaren, 2001; Weissman, 2002). Consensus about the use of residual in vitro fertilization (IVF) pre-implantation embryos is emerging, but the creation of these human blastocysts for therapeutic purposes will probably remain a never-to-be-solved ethical or moral problem, especially in view of the new finding of pluripotency of SS cells from embryonic, fetal, and adult origin.

Whereas new drugs or drug regimes are nowadays studied in humans following the gold standard of clinical pharmacological experiments (CPMP Working Party on Efficacy of Medicinal Products, 1990), the new restorative therapies imply surgical interventions for which formal guidance has not been developed. The new cellular and molecular interventions are usually irreversible types of surgery. None of the approaches has as yet developed into a standard therapy, i.e., they are still experimental approaches. Moreover, since the brain is the site of our mind, which, in interaction with the environment, determines personal identity and personality, the consequences of the putative restorative interventions in the brain must be considered carefully. Each and every new type of restorative brain surgery in PD (and in other neurological, neurodegenerative and psychiatric diseases) has separate safety and ethical issues.

3. How to Approach the Ethical Issues?

There are no guidelines on experimental brain surgery other than those of the general codes for protection of a patient, as set forth, for instance, in the *World Medical Association Declaration of Helsinki* (update 2000), and the “Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine” (Council of Europe, 1997). These and other guidelines deal with aspects of free and informed consent of human beings involved in experimentation (including patients unable to give consent, or those having a mental disorder), the professional standards of the experimental intervention, the risk/benefit ratio of experimentation for the patient, and the obligation for approval by a competent body performing an independent examination on the scientific merits of the study and a review of its ethical acceptability. Guidance on the level and quality of basic knowledge that should validate an experimental surgical intervention, or on the design of the experimental surgery to obtain meaningful and interpretable data, is simply not available. In this respect, the field of experimental (brain) surgery differs from the field of clinical pharmacology, where strict guidance is given, for instance, in the ECC guide *Good Clinical Practice* for trials on medicinal products (1990). Although cell transplantation sometimes has aspects of a pharmacological treatment (if we think of cells as biological mini-pumps of molecules), an irreversible surgical treatment is generally not comparable with reversible drug intake.

Clear guidance is thus not available in the field. Thus, on the one hand, discussion about the ethical issues of new invasive clinical handling in PD

may best be guided by the four general moral principles (Beauchamp and Childress, 1989): 1) human beings and their autonomy should be respected, 2) what is good should be done ("beneficence"), 3) what is bad should be avoided ("non-maleficence"), and 4) what is just should be based on the fair distribution of the available means, on respect for human rights, and on morally acceptable legislation. On the other hand, one may adhere to the rule that any experimental treatment "...must conform to generally accepted scientific principles and should be based on adequately performed laboratory and animal experimentation" (World Medical Association, 2000). This tenant is, however, not very precise. Medico-ethics committees or institutional review boards—nowadays obligatory consultation bodies in all published guidelines for human clinical research—cannot always adequately judge the scientific quality of the animal studies. It is important that scientists themselves discuss the outcome of the fundamental studies as a step toward a clinical trial. The route to human fetal dopaminergic neurografts in PD patients has already undergone such a process (Freed, 1991; Boer, 1999; Peschanski et al., 1999), but other types of experimental, irreversible cell and gene therapeutic approaches in PD are still under debate in the arena of fundamental research.

4. Volume of Preclinical Studies Required

The 6-hydroxydopamine (6-OHDA) nigrostriatal-lesioned rat and mouse models of PD served as the most important and basic test models for putative cell and gene therapies. A more sophisticated model is the MPTP-treated non-human primate, which better mimics the motor disturbances observed in the idiopathic PD patient. Various studies in these models employed dopamine autografts of carotid bodies or adrenal medulla, neural allo- or xenografts of fetal mesencephalic cell preparations, as well as stem cell-derived implants, all placed in the striatum, to ameliorate many of the behavioral deficits caused by the lesion. In these models gene therapeutic strategies to induce dopamine expression or expression of GDNF (glial cell-derived neurotrophic factor) to counteract nigrostriatal degeneration also showed positive results on the primary motor outcomes.

It thus looks like the fundamental ethical requirements in clinical research (a plausible biological mechanism of repair can be presented and a sufficient number of successful animal studies has been reported) have been fulfilled to warrant safe trials on humans. However, this will always remain a matter of dispute as it is difficult to determine how much should be known. What level of pre-clinical research should be required before a clinical trial can be considered? And what measures must be taken to evaluate the benefit/risk outcome of the experimental treatment considering that irreversible surgery is taking place in the seat of the human mind, the brain?

4.1. *Neuronal Supplementation Strategies*

Critical issues for the efficacy of *cell supplementation* strategies are the survival of the implanted cells, their stable dopaminergic phenotype at the moment of and after implantation, and, ideally, their capacity to reconstruct the damaged neuronal circuits. The latter means the occurrence of axonal growth and establishment of appropriate synaptic connections, as well as the reformation of organized input of the new neurons from the recipient brain. Animal PD model studies have not reached complete circuit reconstruction, as dopaminergic neurons had to be placed heterotopically in the (output) striatal area and not homotopically in the substantia nigra for functional recovery (Dunnett and Björklund, 1999). Axonal growth also carries the risk of aberrant connectivity, and this may include the possibility of adverse effects. Recent observations of an enhanced risk of dyskinesia in fetal mesencephalic graft-receiving PD patients indicates that adverse affects can occur (Hagell et al., 2002). The need to revert to animal research to find the exact mechanism that produces dyskinesia and how to prevent this from occurring shows that PD animal models are still valuable for the development of new restorative therapies in human.

The grafting of primary fetal nigra neurons, the implantation of autologous dopamine-producing peripheral cells or neurons, or of dopaminergic cell lines, as well as the placement of pre- or undifferentiated (embryonic or somatic) stem cells, all need to be tested for efficacy in functional repair in PD, and also with regard to safety. These cell implants differ with respect to purity, survival, differentiation, neurite outgrowth and maturation, migration and tumorigenesis aspects, and side effects and may thus differ as much as they do with respect to their efficacy in repairing or replacing the degenerated nigro-striatal system. One would like to see that the critical and safety issues of each of these approaches are subject to evaluation by publication of the rationale and justification of the new approach in the international literature.

The use of human fetal nigra neurons as allografts for the treatment of PD has been reviewed extensively, (e.g., Peschanski et al., 1999; Dunnett et al., 2001), and nobody has challenged the scientific validity of this approach in humans. The same can be said about the use of *porcine mesencephalic xenografts* for the supplementation capacity of these fetal neurons (Isacson and Breakefield, 1997). However, the immune rejection issues as well as the apparent risk of cross-species transfer of viral animal diseases and of zoonosis require higher safety standards than for allografts. These have presently not satisfied well enough to initiate clinical trials (Chapter 11: Barker et al., 2000; Harrower and Barker, 2004). Although some positive results with embryonic and neural (somatic) stem cells have been achieved in the 6-OHDA mouse model for PD, steps toward any clinical trials await a series of fundamental studies on the stability of these cells in vivo, their true neuronal action following differentiation either in vitro or in vivo, and their purification and growth as cell lines for mass production of transplants.

4.2. *Molecular Substitution and Regeneration Strategies*

If each type of cell source for supplementation therapy in PD has its own individual safety aspects, the same must be true for various types of molecular therapy. Whether it concerns the implantation 1) of non-nigra or non-neuronal cells for dopamine release in the striatum or 2) of genetically modified cells for this purpose, or the use of 3) direct gene therapy that is either aimed at replacing the dopaminergic function, 4) at modulating the imbalanced output pathways of the striatum at the level of the STN, or 5) at generating factors like GDNF that may halt the degeneration and/or stimulate regeneration (Dunnett and Björklund, 1999; Kordower et al., 2000; Borlongan and Sanberg, 2002; Le and Frim, 2002), in each case separate background reviews on the rationale and justification for a clinical trial are necessary. Intracranial placement of implants with encapsulated cells that release dopamine or factors to maintain dopaminergic function is yet another approach (Zurn et al., 1996), which in principle may be reversible by removing the device from the brain ventricle or parenchyma. But the chronic exposure to dopamine by introduction of the dopamine-synthetic enzymes or to degeneration-inhibiting/regenerative factors may have side effects as well, since the brain is a very plastic organ in its neuronal circuitry and connectivity.

The critical issues for the implantation of cells for *molecular therapy* are comparable with those for supplementation of dopaminergic (nigra-like) neurons. In addition, however, their post-mitotic character, or, at least, their proliferation characteristics following in vivo placement, should be established. Since the cells have often been genetically modified and insertion of transgenes is relatively random in the cellular genome, the absence of tumorigenicity or the expression of abnormal toxic proteins must be established as well.

Gene therapy, administered either directly on the host brain tissue or indirectly, through implantation of ex vivo transduced cells, raises even more safety concerns as at present it is primarily based on the use of recombinant viruses for gene transfer. Gene transfer to post-mitotic neurons (and other neural cells) has been reached by several classes of viral vectors (Hermens and Verhaagen, 1998). Herpes simplex viral and adenoviral vectors for gene transfer into brain cells must be banned for clinical trials (except perhaps for the killing of a brain tumor), because of the toxicity for several neuronal subtypes. However, the use of adeno-associated viral (AAV) and lentiviral (LV) vectors may be feasible in patients, as absence of toxicity and long-term expression of the transgene are hallmarks for these vectors (Chapter 16: Kordower et al., 1999; Tenenbaum et al., 2003). However, due to the death of patients in clinical trials outside the field of nervous system deficiencies, gene therapy has come under a cloud (Gansbacher, 2003). So, even more than in cell therapy research, safety aspects should be a focus in gene therapy research for PD. Aberrant insertion of the transgene in the genome of the host, the occurrence of integration of virus sequences at uncharacterized integration sites (insertional mutagenesis), as well as the insertion of the transcriptional

active sequences that could result in activation of otherwise silent genes, may have devastating effects on the cells or may lead to disorganization or tumor formation in the nervous system (Connelly, 2002). And this cannot be corrected afterwards. Even the application of co-transduction with killer genes could have traumatic effects due to the inflammatory and immunogenic responses that will occur when activation of this safety system is indicated.

Notwithstanding the above, the results obtained so far in studies of in vivo and ex vivo AAV and LV vector-mediated gene therapy in PD animal models have made it clear that recovery of motor symptoms can be achieved without apparent side effects, and thus offer promise for clinical trials in patients (Freese et al., 1999; Le and Frim, 2002; Kordower et al., 2000; Raymon et al., 1997; Shen et al., 2000). Biosafety of AAV vectors is well accepted, and the use of (Parvovirus family) AAV subtype 2 vector has been cleared for phase I studies in human. The immune system is not challenged by the vector, no toxicity is observed, and inflammatory responses are limited to those related to the surgery. Moreover, the fear that the viral vector, once inside the patient, may recover its ability to cause disease is nil, as AAV2 is not known to be an etiological agent of any disease in humans (Tenenbaum et al., 2003).

During et al. (2001) were the first to present a rationale and justification for the use of AAV vector in the brain. AAV-GAD, a vector that introduces overexpression of glutamate decarboxylase (GAD) for GABA release and when infused in the STN, was able to mimic the therapeutic results of deep brain stimulation effects in the PD animal model. This publication preceded the clinical experimental treatment, which was recently presented as the first and only attempt to put a gene directly into an adult patient's brain in an attempt to treat a neurological disease. As in the case of fetal mesencephalic grafting, it is another example of how researchers take responsibility in the scientific community for the translation of animal-to-human experimentation.

4.3. *Brain as Seat of Human Mind*

Restorative surgery in PD should ameliorate the various handicaps that accompany the disease but without causing severe side effects and without affecting the psyche of the patient in any negative way. The surgery takes place in the brain, the organ of which the activity also reflects the human mind, such as thinking, feeling, perception, will, learning and memory, as well as personal identity. Preclinical studies in animals are not always suitable for evaluating *changes in personality and personal identity* or, in case of neurotransplantation of embryonic or fetal tissue, *personality transfer*, that may occur as possible and unwanted adverse effects (Walters, 1988; HMSO, 1989; Linke, 1993; Birnbacher, 1995).

It is almost impossible to find exact descriptions of personality and identity of a human being in psychology or philosophy that can easily be used in "psychological safety" studies. However, personality and identity are distinct aspects of the individual (Northoff, 1996). Personality is said to be subject to

gradual changes and is scalable, whereas one's identity is unique and immutable. A pragmatic definition for the present purpose would be the following: personality is the complex of mental capabilities and physical performance of an individual, visible to or experienced by others, and absolutely or relatively quantifiable in human behavioral and physical tests. Identity, on the other hand, can be described as the interactively communicated and consistent self-consciousness of a person about his/her past, present, and future (Birnbacher, 1995, Romijn, 1997). In view of current neurobiological knowledge, it seems unlikely that personality or identity is determined by small isolated units of the brain. Characteristics of an individual are more based on the complex interaction of integrated neural networks that are formed by, and based on, numerous nerve cells, often grouped or layered in various sites throughout the central nervous system ("the brain is a collection of individually stupid neurons"). Cellular or molecular restorative neurosurgery of PD patients concerns a local treatment in the brain, and any notion of changes of personal identity, i.e., describing oneself as another person in time and space, are mere science fiction. If personality transfer by fetal neuron transplantation were possible at all, it would require the transplantation of large pieces of intact fetal brain, which, moreover, must be able to survive, to further mature, and to integrate as a network in an existing, fully developed (adult) brain. This is not feasible, as optimal survival conditions are different for each type of cell involved (Björklund, 1992). Physiological characteristics of an individual, however, may be transferable by transplantation, and personality changes (not transfer) cannot be excluded (Arendash and Gorski, 1982; Gage et al., 1984; Ralph et al., 1990; Boer, 1999).

In many cases, PD patients suffer from changes in personality. These changes are either directly or indirectly related to the disease. The physical, psychological, and social situation of patients is indirectly altered by the burden of their symptoms; their limited potentials; fear, depression, and stress; uncertainties about the future; and a possible loss of self-respect and self-confidence. If the burden of the disease symptoms were to be eliminated or alleviated by restorative surgery, many of these personality aspects should improve. The question, however, remains whether, e.g., neurotransplantation of immature neurons or neuroprogenitors will affect personality in an unwanted and irreversible way due to the locally altered morphology and functional cellular interaction in the brain. The aberrant neurite sprouting mentioned above may irreversibly steer neuronal circuitry toward improper functioning. If so, are these behavioral and physiological consequences morally acceptable; or, in other words, do the advantages of the treatment outweigh the subtle personality changes? Answers to such questions can only come from experimental treatments in human. The focus in clinical trials usually is on motor recovery. So far, when investigated in (neuro)graft-receiving patients, no changes in personality parameters have been noticed (McRae et al., 2003) or seen as contraindication (Ostrosky-Solis et al., 1988). However, psychological testing remains necessary for each and every new

(and irreversible) phase 1 study of restorative surgery in PD (and in other brain diseases eligible for this type of therapy).

4.4. Clinical Assessment Protocol

Strategies to control efficacy of various types of restorative clinical trials in PD, is a second translational problem. Human experiments that give non-interpretable results are unethical (Felten, 1994). Shortly after the first clinical trials with intrastriatal implantation of fetal brain dopaminergic cell preparations in PD patients, it became obvious that there was a critical need for a degree of commonality between the methods for patient diagnosis and evaluation by the teams undertaking such treatments. Daily fluctuations make scientific evaluation difficult, whereas the data of different centers had to be compared to achieve sufficient numbers to provide definitive results. An international committee, created in 1990, formulated a series of recommendations for a common and minimum set of diagnostic and methodological core evaluations, called Core Assessment Program for Intracerebral Transplantations in PD (CAPIT-PD) (Langston et al., 1992). It comprises criteria for inclusion of patients, for working definitions and aspects of motor disturbance states, for motor and dyskinesia rating scales (like the UPDRS and Hoehn and Yahr), and time testing of motor behavior, for a pharmacological challenge test, for brain imaging, and, no less important, for a fixed time frame of evaluations to obtain a reliable baseline estimate of the pre-graft clinical status and the post-graft effects for a period long enough for the grafted neurons to mature and become functional. CAPIT-PD has never been completely embraced by the PD grafting field, partly because the program was considered too laborious (and costly) to be carried out in large-scale trials and partly because the grafting was applied as a treatment worth trying rather than as experimental therapy. Had all centers that performed neurotransplantation in PD patients used the CAPIT-PD, a wealth of comparative information could have been obtained instead of the present set of incomparable and seemingly conflicting results. The challenge for the clinical scientist, given the obligation to the test persons undergoing any restorative surgery, is to formulate a CAP that guarantees the collection of objective data on PD symptoms (Boer and Widner, 2002). Objective data are needed to compare pre- and post-intervention data as well as for comparison with a parallel CAP-evaluated randomly assigned reference patient group. Markers of surviving tissue, obtained with imaging techniques, are pivotal for the interpretation of clinical effects and essential for linking clinical effects with a causal mechanism. Within the Network of European CNS Transplantation And Restoration (NECTAR), the CAPIT has been updated and improved to allow comparable evaluation of all kinds of new experimental treatments in PD patients (Core Assessment Program for Surgical Interventional Therapies, CAPSIT-PD) (Defer et al., 1999; Widner and Defer, 1999).

Patient placebo effects and investigator bias will be largely eliminated, or at least minimized, by a series of well-defined quantitative measures of evaluation. Whereas from a strict methodological point of view, randomized double-blind placebo-controlled studies may be the way to go (Freeman et al., 1999), an answer to the question of whether cellular implants in the brain of PD patients are efficacious can then also be obtained with an intra-patient study design that does not involve sham-operated patients (see subhead 7 and Chapter 4).

5. Retrieval and Use of Donor Cells

Cell-restorative surgery in PD began with open trials of striatal placement of the patients' own adrenal medulla tissue (Backlund et al., 1985; Madrazo et al., 1987). This tissue was used experimentally as an alternative source of dopamine neurons in order to circumvent the ethical problems following the use of human fetal brain tissue (Boer, 1994). The cells of the adrenal medulla produce large quantities of catecholamines, for which dopamine is a precursor molecule. Animal models had shown recovery of the experimentally induced motor disorders. However, the clinical results of these non-neural autologous implants were poor, variable, and inconsistent in patients, which was later confirmed in monkey model studies. Though researchers are always eager to develop an effective therapy (Boer, 1996, 1999), this history of adrenal medulla auto-implants in the brain of PD patients illustrates the difficulty of determining what level of prior animal experimentation is required to establish the efficacy and safety of a new clinical treatment (see subheads 2 and 4.1.) Significantly better functional effects in the parkinsonian rat and monkey models with implants of immature dopaminergic nigra neurons made a move toward the use of human embryonic or fetal tissue inescapable (Boer, 1999). Ethical guidelines had to be developed, as the cells were obtained from the remains of human abortions. Nowadays cell-based therapies to replace (or rescue) dopaminergic function in PD use cells obtained from less controversial sources or from sources "cells from the shelf" (Figure 2.1). However, each cell source seems to have its own particular set of ethical problems.

5.1. *Adult Human*

When it had become obvious that adrenal implants in PD had failed, the idea of autologous transplants did not vanish, because of the advantage of avoiding immunological problems and not requiring a donor. Combined adrenal chromaffin/peripheral nerve tissue (Date et al., 1997) or chromaffin/Sertoli cell implants (Sanberg et al., 1997), or treatment with nerve growth factor (Date et al., 1997) to promote long-term survival of chromaffin cells for dopamine production have been studied. Other paraneural cells with dopamine excretion, such as stellate ganglion or globoid bodies, have shown

modest anti-parkinsonian effects (Itakura et al., 1997; Nakao et al., 2001; Arjona et al., 2003). These clinical studies were a corollary of a successful series of rat and monkey PD model studies. The ethical concerns arise from the possible loss of function due to the retrieval of (dopamine-producing) cells from elsewhere in the body (see subhead 6.1).

Yet another process of autologous transplantation is the collection of the patient's own SS cells in order to grow, differentiate, and implant them as dopaminergic neurons. There are no ethical objections to the retrieval as long as the isolation of these cells is a minimal burden for the PD patient which can subsequently be treated or ameliorated for its symptoms.

5.2. *Human Embryo and Fetus—Primary Cells*

Although neural grafting in PD became a real test bed for the possibilities of neurotransplantation therapy in neurological diseases, it also opened the arena for discussion of the ethical acceptability of the use of human abortion remains in the clinic and laboratory. For decades, research on pre-viable and non-viable fetuses and their tissues has been carried out by embryologists and physiologists. These data can be found in handbooks on embryology and intrauterine development (Falkner and Tanner, 1978; O'Rahilly and Müller, 1987) and have contributed to measures that have helped to safeguard early prenatal human life. Thus, in itself, the use of prenatal tissues has not been seen as ethically objectionable (Gareth Jones, 1991). However, the concern arose that the use of embryonic and fetal cells in large groups of patients (not only PD, but also Huntington's disease patients, and patients with haematological, liver, thymic, and pancreatic disorders; McCullagh, 1987) might create a great demand, and thus encourage induced abortions that would otherwise not have occurred (donation as a noble and selfless act for the

FIGURE 2.1. *(caption continued)*

the human. These somatic stem (SS) cells appeared multipotent as well as may also be a source for growing dopaminergic transplants. Embryonic stem (ES) cells present in the inner cell mass of the pre-implantation embryo are easier to grow and expand than SS cells. Their potential as source cells for the preparation of differentiated cells of various organs, including the brain, is great. Hence, dopaminergic grafts may be obtained from "cell lines from the shelf". The pre-implantation embryos could come from the donation of spare embryos from a parents' in vitro fertilization (IVF) program or be created for the purpose of obtaining these ES cells from donated sperm and egg cells from adults (egg cells could perhaps also come from the remains of aborted female fetuses). Finally, an in vitro pre-implantation embryo as a source of stem cells could also be obtained through somatic cell nucleus transfer (SCNT, also called "therapeutic cloning"). The DNA of the patient is then placed in an enucleated donated egg cell, which would provide a method to solve the problems related to immunological rejection of transplants.

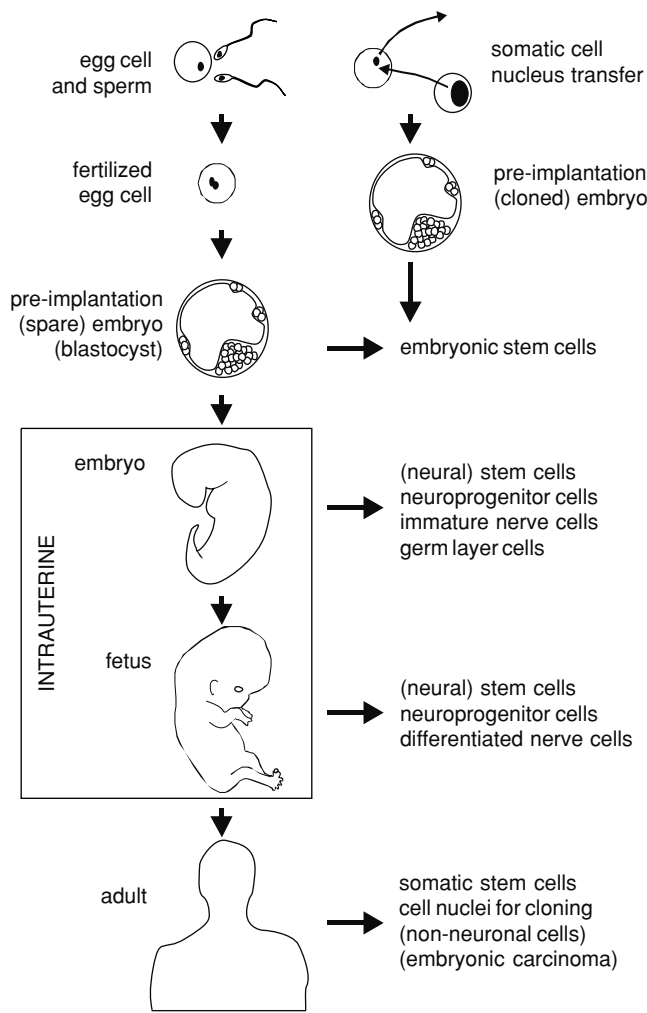


FIGURE 2.1. Present and possible future technical possibilities of obtaining dopaminergic transplants for human neural grafting in Parkinson's disease. The initial steps in experimental neurotransplantation were based on the use of mesencephalic tissue retrieved from human embryos and fetuses, and dissection or preparation of neurografts containing the immature but differentiated dopaminergic nerve cells of the substantia nigra. Several new developments have meanwhile taken place or are foreseeable. First, the embryonic or fetal remains of human abortion could also be the source of neural stem cells and neuroprogenitor cells (stem cells that passed the first stages of differentiation), which can be grown in culture and can be proliferated and differentiated for neurotransplantation. Neural stem cells also appear to be present in the adult brain, but it is unclear whether this can be a source of cells to grow for dopaminergic neurografts. Stem cells are also present in many other organs in the embryonic, fetal, and adult stages of

benefit of humanity). This issue can therefore not be completely separated from the ethical aspects of the decision on elective abortion (Boer, 1994, 1999).

The moral basis of the current legal practice of elective abortion in many countries is that the interest of the woman's physical and social health must be balanced against the interests and viability of (and the respect for) the embryo or fetus in utero, a human being-to-be. Many national and international organizations, national institutes of ethics, as well as for instance the Parliamentary Assembly of the Council of Europe and a Working Group of the European Commission, in addition to scientists in the field themselves (see Table 2.1) have provided ethical guidelines for the subsequent use of body remains for experimental and clinical research (Peel rapport, 1972; CNESVS, 1984, 1990; BMA, 1988; Boer, 1994; De Wert et al., 2002). Despite marked differences, they all aim to solve the above-mentioned ethical problem by trying to achieve complete separation between the decision about abortion and the possible donation of the remains (the so-called *separation principle*) (Boer, 1999; De Wert, 2002). The situation would then be similar to the generally accepted use of organs or tissue from deceased babies, children, or adults. Leaving aside that some give the embryo and fetus absolute worthiness of protection as life, which makes the use of the resultant material

TABLE 2.1. Guidelines for the Use of Human Embryonic or Fetal Tissue for Experimental and Clinical Neurotransplantation and Research (as Formulated by the Network of European CNS Transplantation And Restoration, NECTAR)*

-
1. Tissue for transplantation or research may be obtained from dead embryos or fetuses whose death resulted from legally induced or spontaneous abortion. Death of an intact embryo is defined as absence of respiration and heartbeats.
 2. It is not allowed to keep intact embryos or fetuses alive artificially for the purpose of removing usable material.
 3. The decision to terminate pregnancy must under no circumstances be influenced by the possible or desired subsequent use of the embryo or fetus and must therefore precede any introduction of the possible donation. There should be no link between the donor and the recipient, nor designation of the recipient by the donor.
 4. Neither the procedure nor the timing of abortion must be influenced by the requirements of the transplantation activity when this would be in conflict with the woman's interest or would increase embryonic or fetal distress.
 5. No material can be used without informed consent of the woman involved. This informed consent should, whenever possible, be obtained prior to abortion.
 6. Screening of the woman for transmissible diseases requires informed consent.
 7. Nervous tissue may be used for transplantation as suspended cell preparations or tissue fragments
 8. All members of the hospital or research staff directly involved in any of the procedures must be fully informed.
 9. The procurement of embryos, fetuses, or their tissue must not involve profit or remuneration.
 10. Every transplantation or research project involving the use of embryonic or fetal tissue must be approved by the local ethical committee.
-

*Published on behalf of NECTAR by Boer (1994).

a crime of “complicity after the fact” (Bopp and Burtchaell, 1988), according to these guidelines, even if induced abortion is regarded as unethical, it cannot be concluded that it is inherently wrong to save lives with donated abortion remains (Robertson, 1988; Boer, 1994).

Of course, informed consent should be obtained for the donation. The main additional requirements should be that the timing and method should not compromise efficient medical handling in the interest of the woman and the conceptus, and that no remuneration be involved. The consent procedure and how much one can deviate from abortion procedures in order to obtain useful tissue without violating efficient handling remain open to differences in opinion (Boer, 1999; De Wert et al., 2002). In none of the informed consent procedures of any of the guidelines, has a role been set aside for the sire. In view of the equal legal positions of men and women (in Western societies), a father may have legal rights in this decision, similar to the case of donation of organs from a deceased newborn or child. On the other hand, some women do not want (for personal reasons) to involve the begetter in the process of terminating the pregnancy, or the begetter may be unknown at the time of abortion. These are practical arguments for not routinely seeking consent of the begetter (Boer, 1994) and sticking to consent of the woman. In case of a mutual good parental relationship, the begetter's consent can of course be sought.

Modifications of the method of abortion for the purpose of getting suitable material for transplantation could involve an additional burden for the woman, and thus conflict with her health interests. This objection vanishes for modifications that impose little or no additional risk on the woman. However, postponement of the abortion may be emotionally burdensome as well as being not in agreement with the above-mentioned separation principle (Boer, 1999; De Wert et al., 2002). Adhering to this separation principle has a strategic function, as it ensures that abortion and the use of the remains are separate practices, thereby circumventing any of the accusations of complicity or moral taint as mentioned above. A change in the method does not jeopardize the procedure of separation of decisions.

Although the treatment of PD patients with primary dopaminergic neurons from the donated remains of human elective abortions seems morally and ethically defensible, the therapeutic outcome has been variable. Moreover, successful treatment requires the use and donation of brain tissue from up to 10 abortions, which logistically is an impossible mission. A proof of principle was thus established, but the efficacy of the neuronal supplementation was still poor, if it is to be more successful in the future, it should be based on more readily available cell sources for equal and fair availability for large groups of PD patients. Alternatives are cultured *cell lines*, either grown from SS cells or cells from carcinomas (see subhead 5.1), or proliferated and differentiated from early human embryonic (pluripotent) stem cells, germ line cells, or fetal neuroprogenitor cells (Figure 2.1). The second alternative is implantation of neural grafts grown from animal sources (*xenografting*).

5.3. *Human Embryo or Fetus—Stem Cells*

The finding that the ES cells from the inner cell mass (ICM) of the human pre-implantation embryo at the blastocyst stage can be isolated, propagated, and differentiated into many different types of cells in vitro, including dopaminergic neurons, raised the possibility of growing transplants for engraftment in PD patients (Chapter 13). Theoretically, as these pluripotent ES cells can multiply indefinitely, the ICM of a single human pre-implantation embryo would be sufficient to treat large cohorts of patients (Palacios et al., 1995; Rohwedel et al., 1998; Thomson et al., 1998). However, the precise conditions to achieve and harness cell lines for dopaminergic neuron supplementation are far from established (Deacon et al., 1998; Taylor and Minger, 2005) and research using human pre-implantation embryos remains necessary (and not only in view of the search for cell therapy for PD, but also for diseases like Huntington's disease, leukaemia, stroke, heart failure, diabetes mellitus, burns, etc.). Human cells with the pluripotency of ES cells can be found also as embryonic carcinoma (EC) cells (see subhead 5.1), and as embryonic germ (EG) cells from the germinal primordium of the post-implantation embryo (Shamblott et al., 2001). Furthermore, neuroprogenitor cells from the embryonic or fetal brain can be used as source cells to grow dopaminergic cell grafts. Finally, the SS cells and/or progenitor cells from the embryonic or fetal organs or from umbilical cord blood appeared to have the multipotency to form neural cells. Ethically speaking, the source or donor situation is quite different and requires separate discussion.

5.3.1. Post-Implantation Embryo

When stem cells or progenitor cells are retrieved from the post-implantation human embryo, the situation is similar to when primary neurons are retrieved and used for immediate grafting. The guidelines for donation following elective abortion thus also hold for the use of the remains for isolation and proliferation of neuroprogenitor, SS, or EG cells. Two main aspects are different. Informed consent must, of course, be obtained for a different research goal: the establishment of a cell line for research or possible cell therapy. Secondly, screening of transmissible diseases in the individual donating the cells can be omitted as safety tests can be performed on the cultured cells.

5.3.2. Surplus Pre-Implantation Embryo

So far, the potency of mammalian ES cells for expansion and differentiation appears to be superior to that of SS and other types of pluripotent or multipotent cells. There is thus a strong scientific wish as well as a need to explore ES cells from human blastocysts as source cells in order to grow transplants (see also subhead 5.5). The creation of human blastocysts is a current practice in IVF programs that help to fulfill the desire for parenthood of couples with particular reproduction problems. In the research preceding this type of

treatment of infertility, human blastocysts had been created as a means to an end of developing the methods. The *in vitro* creation and sacrifice of human pre-implantation embryos is still mainly limited to research in the reproductive field (infertility treatment, causes of congenital diseases and miscarriage, and improvement of techniques and quality of IVF). It is licensed under strict control of regulatory bodies in some countries and completely forbidden in others. This is indicative of the differences in moral views of human embryo *in vitro* pre-implantation in different societies.

Due to the burden of the necessary hormonal treatment, current practice in IVF protocols is that the collection of egg cells is performed once. After IVF and some days of growth and quality control in the dish, blastocysts are then implanted in the uterus for pregnancy or deep-frozen for a second trial or subsequent pregnancies. At some point the latter are no longer necessary and regarded as “surplus” embryos (also called rest, residual, spare, or supernumerary embryos). Although extreme pro-life activists claim that these human-beings-to-be must not be destroyed and can only be thawed for implantation in a womb, pragmatism leads to destruction. If the use of the remains of an aborted post-implantation embryo can be ethically justified, the use of “surplus” embryos cannot be rejected. The protection of the *in vitro* human blastocyst, a liquid-filled tissue sphere with undifferentiated cells (Scothorne, 1968; Singer, 1990) should not be held in higher esteem than that of an intrauterine human embryo in which organogenesis into a visible human-being-to-be has taken shape. It reflects the principle of relative worthiness of protection of an embryo, which gives increasing protection as intrauterine development progresses (HER, 1994).

Many, but not all, of the guidelines developed for the retrieval and donation of the embryonic or fetal remains after elective abortion (Table 2.1) are of value also for the donation of “surplus” embryos. The decision not to implant opens the door for donation. The removal and cultivation of cells from such otherwise destroyed pre-implantation embryos could be regarded as analogous to tissue donation after elective abortion. In these cases either being conception *in vivo* or *in vitro*, the use of embryo cells is not intended *a priori*, and consent for donation is sought for the use of the remains, albeit at different embryonic ages. The IVF couple (not just the woman) should provide informed consent, the separation principle should be maintained (no IVF embryo created nor designated by the couple for a particular patient), and no elongated *in vitro* growth of the intact blastocyst toward post-implantation developmental stages should be sustained (Table 2.2).

5.3.3. Pre-Implantation Embryo—Creation and Therapeutic Cloning

Whereas the use of “surplus” embryos largely corresponds with the use of remains following elective termination of pregnancy, the use of blastocysts created solely for experimental or therapeutic use introduces a new moral problem. Here human life is created for the purpose of sacrificing it as a cell

TABLE 2.2. Proposed Points of Guidance for the Use of “surplus” IVF Embryos for Experimental and Clinical Research Toward Stem Cell-Based Cellular Therapies

-
1. It is not allowed to keep embryos alive artificially for the purpose of reaching the developmental stage of an in vivo post-implantation embryo (upto 10 days post-fertilization).
 2. The decision to destroy so-called “surplus” embryos must not be influenced by the desired use and must precede the question of possible donation. There should be no link between the donor couple and the recipient, nor designation of the recipient by the donor except for possible treatment of the IVF offspring of the couple.
 3. No material can be used without informed consent of the gamete providers involved.
 4. The procurement of “surplus” embryos must not involve profit or remuneration.
 5. All members of the hospital or research staff directly involved in any of the procedures must be fully informed.
 6. Every research project involving the use of rest IVF embryos must be approved by the local ethical committee.
-

bank to grow therapeutic cell lines. Moreover, the use of the created embryo does not improve the quality of future in vitro procreation and, hence, the quality of life for persons conceived this way, i.e., does not future IVF children (McLaren, 1996). For restorative cell surgery, the creation of the human blastocyst must be regarded as a pure instrumental use of a human-being-to-be, and so a re-evaluation of its ethical acceptance is needed.

Pre-implantation embryos can be created not only with donated gametes, but also through somatic cell nuclear transfer (SCNT) in an enucleated donated egg cell (Wilmut et al., 1997; Cibelli et al., 2001). So far in non-human mammalian species only, since the report on successful hSCNT (Hwang et al., 2004) has recently been withdrawn (Normile et al., 2006). SCNT has been called therapeutic cloning, in order to distinguish it from reproductive cloning (which is forbidden almost everywhere). Therapeutic cloning has the advantage of creating a human blastocyst that contains ES cells of the genotype of the patient eligible for cellular therapy. It circumvents the problems of tissue rejection by the recipient and saves the patient from the unsatisfactory and troublesome life-long treatment with immunosuppressive drugs (Wolf et al., 1998).

In the many publications on the above-mentioned issue as well as in the public and political domains, the creation and use of human pre-implantation embryos for therapeutic purposes is a controversial issue that is unlikely ever to reach consensus. Societal and religious views on the human value of and respect for human life at the early stages of morula (4–5 days) and blastocyst (5–10 days after fertilization) simply differ too much (De Wert et al., 2002; Matthiesen, 2002; Oduncu, 2003). For many it is difficult to maintain moral principles that forbid the creation of the primitive stages of an embryo, if its cells may be an endless source of great therapeutic potential for a variety of severe or life-threatening diseases of adults. This includes PD patients for whom there is otherwise no satisfactory cure (see also subhead 5.5).

Hansen (2002) even argued that the moral status of a blastocyst created through SCNT is “found to be more clearly not equivalent to that of a human being” than a blastocyst created by gametes. Fewer ethical problems for the collection of stem cells in the former case therefore arise. For the time being, some form of consensus seems to have been reached to first make use of surplus human pre-implantation embryos to develop methods and prove efficacy in the patient (McLaren, 2001; Outka, 2002). When it has been shown that the creation of pre-implantation embryos is really needed, perhaps the temporary ban could be lifted.

Some fundamental rules, however, remain of importance: the providers of the gametes to perform IVF or SCNT must donate under the regime of informed consent for the purpose of the creation of the pre-implantation embryo, and donation must under no circumstances be obtained under pressure or for remuneration. Moreover, permission from a local medico-ethics committee should be obligatory (McLaren, 2001; Nuffield Council on Bioethics, 2000, Dutch Health Council, 2002).

5.4. *Animal*

In view of the limited availability of human embryonic or fetal tissue, xenogenic tissue is seen as an alternative tissue for grafting (Chapter 11). Animal nerve cells make use of a similar molecular repertoire to serve very similar functions of cellular communications and activity responses in the brain as human nerve cells (Isacson and Breakefield, 1997). Moreover, human neuroprogenitor cells transplanted in the germinal ventricular zones of the postnatal developing rat brain take part in rat brain development as if they were rat cells (neurons are formed that migrate and settle in a network of genuine rat cells; Flax et al., 1998). Xenografting makes use of this chimeric plasticity of undifferentiated or immature mammalian nerve cells. Indeed, pig fetal mesencephalic grafts placed in rat models for neurodegenerative diseases like PD exhibit allograft-like morphology and a remarkable axonal target specificity as well as a functional restoration of impaired motor behaviour (Huffaker et al., 1989; Isacson et al., 1995; Galpern et al., 1996). For scientific, practical, and ethical reasons, in particular, embryonic tissue from porcine origin is being investigated as a neural graft (Dunning et al., 1994; Advisory Group on the Ethics of Transplantation, 1996; Nuffield Council on Bioethics, 1996; Daar, 1998). Pigs have been selected because of their brain size, and because there is extensive experience in large-scale breeding of these animals for food. Also there are ongoing attempts to apply porcine organs for transplantation in humans.

The ethical discussion in xenografting covers the welfare and choice of animals as source of transplants, as well as the dangers of infections and long-term immunosuppressive treatment of the patient, and the psychological acceptance by the recipient. Many of these points have also been discussed in

view of the shortage of organs for patients suffering from end-stage organ failure (Nuffield Council on Bioethics, 1996). Animal protectionists challenge and oppose the use of specially bred animals as a source of transplants, since the special breeding conditions—necessary to control the pathogen status of the source animals—would introduce yet another violation of animal integrity and autonomy, and would compromise animal welfare in a new type of factory farming. Ethically speaking, there should be no difference between breeding animals for food or breeding them to harvest cells or organs for transplantation (Daar, 1998), providing that suffering can be kept to a minimum. With all due respect for the life of animals, the integrity and autonomy of a pig should not be viewed on a human level. One might even say that breeding for transplants serves a higher goal. Any validation of welfare should, however, be weighed against the potential benefit to patients, and animals used for animal-to-human transplants are to be protected by animal acts.

The greatest concerns connected with xenotransplantation is that of the possibility of pathogens', specifically viruses and prions, jumping the species gap (Butler, 1998) and the necessary combination with life-long immunosuppression treatment (see subhead 6.2). These problems are absent when genetically modified animal cell lines are used, encapsulated in semi-permeable polymers for the local release of neuroregenerative or neuroprotective proteins in the nervous system. Cells from lower animals or even invertebrates can even be applied with no ethical objections against the source from society.

5.5. *Proportionality and Subsidiarity*

Possible objections on the use of human embryos for research and therapy are also connected to the ethical principles of proportionality and subsidiarity. The *principle of proportionality* is translated as follows: the use must serve an important goal in the interest of human health. It is difficult to claim that isolating cells from human abortion remains or from “surplus” or created pre-implantation embryos is disproportional. In many societies elective abortion is legally accepted, pre-implantation embryos have been and still are used for research into the causes or treatment of infertility. It would be inconsistent to reject research on cell replacement therapies that may lead to treatment of severely handicapping and yet untreatable diseases like PD.

The *principle of subsidiarity* in restorative surgery with human cell implants implies that the goals of research or application cannot be reached with alternative sources for suitable cells other than the human blastocyst, embryo, and fetus, or cannot be reached by other methods than cell therapy. First of all, one has to realize that research on cellular replacement therapy is presently focusing on the establishment of cell lines for transplantation. An endlessly proliferative cell line with the capacity to form dopaminergic neurons would theoretically eliminate the need to obtain new material for separate PD patients (“dopaminergic transplants from the shelf”). This is not only true for the hES

cells as a source cell from pre-implantation embryos, but also for the pluripotent hEG cells and the multipotent neuroprogenitor cells from human embryos and fetuses. Calling a halt to this research would obstruct important new medical developments in embryo-use-saving methods that could moreover be applied in larger cohorts of patients. However, the finding that pluri- and multipotent stem cells are everywhere in the human body, also in adulthood, raised new criticism from people who do not accept the use of human embryonic and fetal sources (Oduncu, 2003). SS cells (also called "adult" stem cells) have now been isolated from bone marrow, liver, skin, fat tissue, umbilical cord blood, and the CNS. Their potencies seem remarkable and they are described as "blood into brain, brain into blood cells" (Bjornson et al., 1999; Brazelton et al., 2000; Mezey et al., 2000; Toma et al., 2001). The message that the use of cells from human blastocysts, embryos, and fetuses should be banned hampers new developments as well. However, recently the capacity of SS cells to transdifferentiate has been questioned, and the moment, the potencies of proliferation and differentiation of ES cells are superior to those of SS cells. Limiting the field to the use of SS cells would delay research in clinically operational and efficacious cellular treatments in PD (and many other organ-failure diseases). So, although the principle of subsidiarity is meant to express concern for the moral value of the embryo, it is a sign of ethical one-dimensionality to present every alternative which does not use early human embryos *a priori* as being morally superior (De Wert, 2002). Xenografting as an alternative for the use of allografts is at present no alternative either. From the perspective of animal ethics, one may question whether it is reasonable to breed and kill animals in order to obtain transplants when, e.g., residual human IVF embryos can be used that would otherwise be discarded.

Cell supplementation therapy is just one route to restorative surgery in PD. Logically, the homotopic placement of new dopaminergic nigra neurons that develop the proper output and input connections following implantation would be ideal (Dunnett et al., 2001). However, the principle of subsidiarity should also be considered with respect to other methods that may have a comparable therapeutic effect, rather than restoration of lost parts of the nigro-striatal system. Deep brain stimulation, thalamic lesions (Benabid, 2003) or chronic delivery of nigra-protecting GDNF (either via infusion, delivered from encapsulated cells, or from genetically modified autologous cells) (Kordower et al., 2000; Gill et al., 2003; Slevin et al., 2005) are therapies that are in various stages of clinical development. New approaches may be envisaged as well, such as *in vivo* stimulation of neurogenesis in the substantia nigra (Zhao et al., 2003). However, none of these methods can be seen as a standard treatment for PD, and outcomes never fully ameliorate all aspects of the disease symptoms. So, all these strategies need to be developed in parallel. The variability in the disease phenomena of PD patients may even require a repertoire of restorative and symptom-correcting methods for truly efficacious treatments.

6. The Recipient Patient

Animal “models” for PD are only of relative value because they do not mimic the disease in terms of long-term chronic neurodegeneration nor do they completely match the symptomatology and prognoses in the human situation (Bankiewicz et al., 1993). Final efficacy of any neurotransplantation approach will thus need clinical trials, with the risk of negative effects in human beings. In general, one may say that a new experimental approach should be presented in written form to a scientific committee of experts in the field, who should evaluate the proper step towards human experimentation as well as the study design. The ethical principles of value and scientific validity for initiating such trials are generally regarded to be fulfilled if immature nigra neurons are to be used (see subhead 3). For other types of cellular and molecular restorative surgeries in PD a decision-to-go has been taken or is still under discussion. Fetal nigra-containing grafting in PD patients has in fact been a first test bed for all of the new types of restorative neurosurgery, and has initiated ethical discussions on the conditions, source, and donation of the cells to be implanted (see subhead 5). However, an ethical evaluation concerning favorable risk-benefit ratio, informed consent, and respect for the patient involved in the study should be considered as well.

6.1. *Informed Consent*

It goes without saying that participation in any restorative surgery project must be voluntary and that there is a right to withdraw consent at any time (United Nations, 1948; Boer, 1994; BMA, 1988). PD patients, though vulnerable, are still able to understand their situation. They are therefore regarded as competent to give informed consent to be a subject in experimental therapies. In advanced stages of the disease their mental state is often—but not always—impaired, but not to such an extent that it affects their communication, understanding, and expression of free will (Koller, 1987). The assessment of competence/decision-making capacity is the primary responsibility of the investigator. As a general rule, a candidate subject can be considered competent when the nature of the information, the consequences and risks of being a research participant, and the possibility to refuse are understood. Considering the fact that invasive irreversible experimental surgery is involved, a second opinion on the assessment of competence/decision-making could be sought from a physician who is not involved in the research project. Some patients may have difficulty in expressing choice, and others may be unduly susceptible to the harm and stress of being a research subject. Researchers could minimize this vulnerability by including family members or patient representatives in the decision-making process. Oral and written information should be provided. Moreover, informed consent alone can never be an argument to initiate clinical experimentation. Putative efficacy, biosafety, and experimental design are equally important.

6.2. Biosafety

Biosafety has multiple facets, as there are various types of experimental restorative neurosurgery in PD. Human fetal primary brain cells as striatal implants will raise other safety concerns than the application of viral vector-mediated gene therapy in the nigro-striatal pathway, or xenografting combined with immune suppression treatment.

6.2.1. Transmissible Diseases From Human Transplants

Testing for transmissible human diseases of the donor tissue is a necessity. In case of a short time interval between the retrieval of the transplant from human abortion remains and the actual surgery, the tests can only be performed on the blood of the woman. These tests should thus be performed prior to the elective abortion, which requires her separate consent (Boer, 1994). This is routine procedure in all existing protocols of neurotransplantation involving embryonic mesencephalic tissues. The recently adopted EC Directive of the European Parliament and Council on setting standards of quality and safety for the donation, procurement, testing, processing, storage, and distribution of human tissues and cells (2003), requires serology tests for HIV1 and 2, hepatitis B and C, *Treponema pallidum*, and HTLV-I and II. Storage and hibernation of the abortion remains make it possible to carry out these tests without blood sampling from the woman. However, so far, for primary cells, this is done at the expense of neuronal cell survival following grafting (Chapter 8, Frodl et al., 1994). Contaminations contraindicate the direct use for implantation.

The above tests are of course also indicated when allogeneic ES, EG, fetal neuroprogenitor, or SS cells are to be used to grow dopaminergic transplants in vitro, or when cell lines from human embryonic teratocarcinoma are to be applied. However, the cell cultures can be tested for pathogens during the proliferation, differentiation, and storage phase before final use.

6.2.2. Zoonosis Following Xenotransplantation

The greatest concern connected with xenotransplantation is that of the possibility of pathogens, specifically viruses and prions, jumping the species gap (Butler, 1998). Animal viruses could be transmitted to humans (zoonosis), as evidenced by the large disease outbreaks in humans of the Ebola and Marburg monkey viruses, of the simian-derived HIV AIDS virus, and more recently of bird flu (Webster et al., 2005). Barker et al. (2000) formulated a series of specifications that should be fulfilled. They comprise microbiological specification of the pig strain, biosecurity of animal production, sterile tissue collection, creation of a tissue archive and safety database, and an investigation of porcine endogenous retroviruses (PERVs). Even assuming that the use of domestic pigs—which have been in contact with humans for ages—as source animals for cells will be less dangerous, zoonosis cannot be

completely avoided by pathogen-free breeding. PERVs are integrated into the genome, as are retroviral DNA sequences in the human genome and other mammalian species (Weiss, 1998). Zoonosis could therefore also be a result of DNA recombination and adaptation, leading to the expression of known or newly formed retroviruses. Though not directly pathogenic for humans, pathogenicity of porcine viruses can change unpredictably when they cross species. The chance of cross-species infection (Patience et al., 1997; Martin et al., 1998) increases with the closeness of contact of grafted and host cells following neurotransplantation, and the reduced competence of the immune system of the immunosuppressed graft-receiving patient. In a worst-case scenario, xenotransplants could introduce a very infectious, or possibly lethal pathogenic virus that would not only affect the graft recipient but could also (through human-to-human contacts) lay humanity open to a new plague (the Trojan xenotransplant) (Butler, 1998; Bach et al., 1998). To the xenograft recipient, the benefit of a successful transplant will certainly outweigh the risk of any subsequent unwanted effect of infection by a pig virus. To society, however, the possibility of setting off a new human epidemic requires fundamental virology studies before any ethical judgements are passed.

So far, patients who have received pig organ or tissue transplants (Nasto, 1997; Stoye et al., 1998; Heneine et al., 1998) or who were dialyzed with pig kidneys (Patience et al., 1997) have shown no signs of porcine virus-induced pathogenesis. Clinical studies with porcine embryonic mesencephalic dopaminergic grafts in the brain should still include long-term post-operative screening on the expression of porcine endogenous retroviruses in serum samples (Isacson and Breakefield, 1997). Possible consequences for the patient when hazardous viruses do show up have hardly been considered in the neurotransplantation field. If it affects just the patient, it is to be regarded as side effect. If it becomes a highly transmissible, life-threatening disease, at the extreme it could mean isolation of the xenotransplant-receiving person.

6.3. *Risk/Benefit Aspects*

Experimental restorative neurosurgery is not without risk for the PD subject involved (Boer, 1999). In all cases mentioned in head 2 this type of surgery involves intracranial manipulations after drilling a hole in the skull, either under local or complete anaesthesia. The risks of the surgery, up to opening of the skull, are relatively mild. The risks involved in going into or through the brain parenchyma are more severe, since, despite extensive pre-clinical studies, the dangers of placement of cells are sometimes not fully predictable. However, the ethical principles of beneficence and non-maleficence must prevail at all times (see subheads 3 and 4), and a net therapeutic outcome should always be the expectation.

Clinical neurotransplantation in PD subjects has so far been carried out either with autologous chromaffin cell-containing tissue, or with mesencephalic allo- or xenografts obtained from, respectively, electively aborted

human fetuses or specifically bred pregnant pigs. Sterile handling and tests for transmissible diseases prior to implantation (see subhead 6.2) are the first measures for safety, and can be seen as routine in any transplantation surgery. Adrenal medullary tissues as an autologous graft source for the chromaffin cells were abandoned as cell survival is poor and—in some centers this approach was regarded as a therapy rather than experimental treatment—morbidity and mortality were the side effects. Using human fetal mesencephalic grafts in PD, researchers have only recently identified the side effect of uncontrollable hyperkinesias in “off” phases (periods of increased motor disability) (Freed et al., 2001; Hagell et al., 2002). Here one has to go back to the animal research to investigate the cause before new, adapted—if feasible at all—clinical trials can be initiated. The benefit of fetal mesencephalic grafting is said to be limited by poor dopaminergic neuron survival and insufficient integration and reinnervation within the deficient striatum of the PD patient (Brundin et al., 2000). This is certainly the case for porcine xenografts, the clinical trials with which were apparently performed too early, as in rodents; e.g., cyclosporin A-mediated immune suppression protects porcine grafts only during the first weeks and fails in the long-term (Larsson et al., 2001).

Transient psychotic symptoms have been reported in some cases of mesencephalic engraftment in PD subjects (Freeman et al., 1999). Safety concerns regarding personality transfer or changes (even emphasized for porcine grafts; Linke, 1993; Coffman et al., 1998), and of negative alterations in cognitive or mental functioning have not been reported. In fact, they were not to be expected as the approach was a cautious one, with only minute volumes of cell suspensions or small fragments used for grafting (see subhead 4.3). The risk/benefit ratio is thus neither uniformly low nor very high, but at the present stage fetal mesencephalic grafting should not be recommended as a therapy. The findings should, however, not block further trials, as significant recovery is seen in many patients, and harmful hyperkinesias in the “off” state are not frequently seen and may depend on the particular transplantation protocol applied. In fact, the results so far must be seen as encouraging for other types of restorative neurosurgery because of the relatively mild, or at least infrequent, signs of harm in comparison with the severe incapacitating symptoms in PD (non-maleficence). Critical evaluation of the preclinical results in animal PD models, published as a review, that deal with efficacy on motor recovery in relation to the morphology and function nigro-striatal pathway, and with such safety aspects, must be seen as a prerequisite (see subhead 4).

The safety aspects of molecular restorative treatments using AAV viral vectors for the overexpression of GAD for enhanced GABA release in the STN have been dealt with (see subhead 4.2), and here one has to see whether the expectation of a low risk/benefit ratio will hold in the first current clinical trial (During et al., 2001). For any other approach, whether it is the application of cografed cells supporting the mesencephalic dopaminergic transplants, implants of immortalized cell lines with dopaminergic phenotype, paraneural

cell implants that secrete neurotrophic or growth factors to halt nigro-striatal degeneration, or direct gene therapy for trophic factors to rejuvenate the system (Kirik et al., 2004), the risk/benefit aspect still has to be discussed, as do the definitive answers on efficacy, preferably in PD non-human primate models. This certainly is the case for ES-cell-derived transplants in PD subjects, for whom the way from hype to hope for an “dopaminergic neuronal transplant from the shelf” is very long (Chapter 13).

7. Experimental Design

Clinical neurotransplantation in PD began with open trials of striatal placement of the patient’s own adrenal medulla tissue (Backlund et al., 1985). The studies immediately raised the question of whether enough basic studies had been performed to justify such an experimental clinical treatment. The team that performed the first stereotaxic neurotransplantation in PD argued that the frustrating lack of treatment for advanced PD patients had weighed heavily in their considerations, and that the respective national and university-based ethical committees agreed to start this enterprise (discussions at the Eric K. Fernström Foundation Symposium on “Neural grafting in the human CNS,” Lund, Sweden, 18–22 June 1984). The outcome of this first study was negative with respect to efficacy. Later, Madrazo et al. (1987) reported significant benefits, but the (afterward overenthusiastic) interpretation of trial data could not be replicated by other groups, and the method was therefore primarily a failure (see subhead 4). This history of adrenal medulla auto-implants in the brain of PD patients illustrates the difficulty of determining what level of prior animal experimentation is required to establish the efficacy and safety of a new clinical treatment. It also illustrates that a move toward the first clinical trial is often prompted by the absence of any effective treatment (Boer, 1996, 1999). Finally, it stimulated discussion on the need for an experimental design for such open-label studies that would give interpretable results. The “birth” of CAPIT-PD was the result (see subhead 4.4). CAPIT-PD improved patient selection, with reproducible pre-treatment measures of the motor scores as baseline, a 1–2-year follow-up period with fixed protocols for read-out of motor scores, and graft survival as key elements.

The value of CAPIT-PD was recently further established by a systematic review and meta-analysis of its measures from study groups that embraced this protocol in their mesencephalic grafting studies (Polgar et al., 2003). Consistent trends demonstrating recovery on many outcome measures could be identified, and recommendations for best practice reported. Ethically speaking it should be highly recommended that researchers put their standardized acquisition data in a CAPIT-PD database, as this would allow a more powerful statistical analysis for evaluating the overall benefits and harm of any type of reconstructive neurosurgery in PD. It saves many subjects from undergoing experimental surgery.

7.1. *Double-Blind Sham-Controlled Studies*

The variable outcome among PD subjects following neurografting has led to the notion that results may be influenced by placebo effects and investigator's bias. To investigate further the efficacy of this new medical technique, double-blind, sham-surgery-controlled studies were proposed and initiated. Sham surgery is a completely new approach in restorative surgery, which had never received a critical evaluation in scientific literature until Freeman et al. (1999) published a plea in favor of this approach in experimental cell supplementation therapy in PD. Organ transplantation cannot be performed with sham surgery ("take the heart out and put it back") to control for bias or placebo effects, but adding cells by injection could in principle be performed under the rules of the gold standard of pharmacological studies. Placebo effects are not uncommon in the clinic, nor in PD studies (Shetty et al., 1999; Goetz et al., 2002), and investigator bias with overenthusiastic interpretations leading to surgeries or drug treatments that were later abandoned for lack of efficacy are not uncommon in clinical science (Albin, 2002). Moreover, De la Fuente-Fernandez and Stoessl (2002) found an effect of placebo administration on striatal dopamine release in PD subjects, and suggested the release was related to signal expectation of reward. Freeman et al. (1999) pleaded for sham (imitation) surgery under the following three conditions: 1) it should address an important research question that cannot be answered by a study with an alternative design that poses a lower risk to the subjects, 2) there must be preliminary but not conclusive evidence that the intervention is effective, and 3) the treatment should be sufficiently developed so that it is unlikely that it will become obsolete before the study has been completed. The latter two criteria pose no problems, as it is generally accepted that neurotransplantation surgery can be effective; there is need for improvement and perfection, but adverse effects are not too prominent (see subhead 6.3) (Brundin et al., 2000; Dunnett et al., 2001). The criterion for discussion is, therefore, the first one.

Sham controls were introduced with full-blown pre- and postoperative patient assessment and surgery, except that the dura was not penetrated and no needle insertion was performed (Freeman et al., 1999). The procedure is thus no control for the implantation surgery, but only controls for the placebo effects of the event of the surgery and of the pre- and postsurgical evaluations. At the outset, with the initiation of such studies meanwhile finalized and reported on (Freed et al., 2001; Olanow et al., 2003), critics claimed that it was too early to include large-scale sham-controlled studies with 30–40 patients when it was obvious that dopaminergic neuron survival was insufficient and transplant methods far from optimal (Widner, 1994). Moreover, surgical implantation of cells in the brain cannot simply be compared with a pharmacological treatment for which extensive guidelines are published, and a double-blind, placebo-controlled design is the gold and often legally required standard (CPMP Working Party on Efficacy of Medicinal Products,

1990). In pharmaceutical research, placebo is not known to produce any adverse effects, and subjects on the placebo do not risk positive harm (harm of commission), but only the harm of omission or exclusion (London and Kadane, 2002). Sham surgery carries risks, and it is thus undeniable that performing surgery with no potential benefit fails to minimize the risk of positive harm. The highest standards of research design were said to be in conflict with the ethical standards of beneficence and non-maleficence (Macklin, 1999). However, this ignores the fact that research ethics take into account not only the interests of the research subjects but also the interests of biomedical research, of the category of patient (aspirational benefit in contrast to direct or collateral benefit for the study subject), and of society at large (Dekkers and Boer, 2001; Albin, 2002). Freeman et al. (1999) stated that these risks are “reasonable” in relation to the possible benefits (sham patients undergo one imitation surgery and will then be elected for an effective treatment later on). Thus, if the chosen research question cannot be answered by a trial that poses fewer risks to subjects, then the use of a sham-surgery control may be a necessary component of a sound clinical trial. It is precisely this which remains the controversial issue: the alleged imbalance between the risks to subjects of the placebo group versus potential benefits to them and to society as a whole. Advocates argue that placebo surgery reduces the bias and increases the objectivity in result analysis, which would protect the public at large from a potentially dangerous procedure being available as a result of incorrect interpretation of test results. The latter points are therefore the crucial ones to consider in determining whether sham neurosurgery is acceptable and whether solid answers to a restorative neurosurgical research question can be obtained without sham control.

The plea against sham-surgery controls by Macklin (1999), London and Kadane (2002), and Clark (2002) is mainly based on the notion that researchers use individuals as a means-to-an-end for the study. Sham surgery is said to violate principles of respect for the autonomy, beneficence, and justice, whereas the validity of free and informed consent from the patients is undermined by therapeutic misconception. However, the lack of consensus on this point in itself may not be an argument against sham surgery. Lack of consensus has not prevented the first clinical neural grafting trials in a balanced, fairly discussed, and ethically acceptable fashion. If there is no other study design that will provide interpretable data, sham-controlled studies may be acceptable (Freeman et al., 1999; Dekkers and Boer, 2001; Albin, 2002). A significant number of PD patients worldwide have received an implant without evidence of a long-lasting therapeutic effect, whereas there is compelling evidence that the clinical course of the engrafted PD patients parallels the development of the graft measured with the surrogate marker F-dopa uptake in PET scans (as animal studies predicted) (Lindvall, 1999; Piccini et al., 1999; Dunnett et al., 2001). Following this, imitation surgery may not be needed, an aspect underestimated in the line of thinking in favor of sham surgery by Freeman et al., (1999). The imitation-surgery control

patients of the double blind studies indeed revealed no improvement of motor ratings (Freed et al., 2001; Olanow et al., 2003).

The necessity of sham-surgery control was additionally challenged on the basis of the so-called principle of equipoise (London and Kadane, 2002). In pharmacological studies, two drugs may be compared in a gold standard procedure to see whether a new compound is equally or more effective than an existing compound of proven effectiveness. The equipoise states here that there is no need for any patient group to be knowingly sacrificed in terms of welfare or body integrity. For PD patients there are pharmacological treatments available, albeit not sufficiently able to cure the symptoms in the late stages of the disease. Comparison of old and newly treated patient groups is thus possible. But this requires a solid standardized core assessment protocol, CAP-PD, with measures that are as objective as possible.

7.2. *Plea for the CAP*

Human experiments that gave non-interpretable results are unethical (Felten, 1994), but so are unnecessary control treatments in human beings. Sham surgery should not be the default control method (Albin, 2002). However, this requires the use of a solid standardized CAP-PD, based on quantitative measures, and on a rigid schedule running from the pre-operative period through to post-convalescence (Boer and Widner, 2002). The latter is important, as it has been shown that it takes years before the full effects are reached following, e.g., human fetal nigra grafting. The application of a maximally objective CAP guarantees that data are collected that can also be compared with a parallel CAP-evaluated randomly assigned reference group or to groups that are subjected to other surgical interventions. Objective markers of surviving tissue, using imaging techniques, should also be recognized as pivotal for the interpretation of the clinical effects and should be used to link the clinical effects with a causal mechanism (Brooks et al., 2003). Negative scans combined with a modest clinical improvement indicate non-cellular therapy or non-molecular therapy-derived effects.

Other experimental irreversible surgical interventions in PD, such as placement of electrodes for deep brain stimulation (DBS) (Benabid et al., 1991; Benabid, 2003) and strategic lesions in the outflow pathways of the basal nuclei (Tasker et al., 1983), or gene therapeutic interventions to enhance the insufficient dopamine release (During et al., 1998) to modulate the output pathways in the STN (During et al., 2001) or to stop the degeneration of the dopaminergic nigra cells (Zurn et al., 1996; Kordower et al., 2000; Gill et al., 2003), would also require a proper unbiased control method to determine long-term efficacy and the absence of severe side effects. A validated PD-dedicated CAP would then be most valuable as an evaluation instrument, even though specific aspects may have to be skipped or added (e.g., repeated PET scans to determine dopaminergic function may be less effective when lesion treatment is applied). A maximally objective CAP-PD remains of value for open trials, too,

as pre- and post-treatment measures can be compared satisfactory (Boer and Widner, 2002).

8. Summarizing Remarks

The field of restorative neurosurgery in PD is booming. A variety of restorative cellular and molecular neurotherapies are under investigation ever since a proof of principle for (partial) recovery of the PD motor disturbances was reported following human fetal mesencephalic grafting in the caudate/putamen complex of PD subjects. These outcomes validated the rationale behind the approach: dopaminergic neuronal input in the striatum can be restored, and the decreased motor anomalies of the patient relates to neuronal cell survival and dopamine release. These results ethically justify further clinical experimentation in neurotransplantation surgery using embryonic or fetal tissues containing the immature dopaminergic neurons. These tissues, available after legislated or legal elective abortion, may be retrieved under two main conditions: 1) donation with informed consent after the definitive decision on abortion and 2) no remuneration or designation of the recipient patient (the separation principle). The variability and limited efficacy of cellular supplementation with immature mesencephalic dopaminergic neurons, as well as the logistical problems to compile (or store) sufficient disease-free and homogeneous cellular material for grafting have led to the search for alternatives that repair the degenerated nigro-striatal system.

Porcine xenografts, autologous transplants of adrenal medulla chromaffin cells with support of co-transplanted cells or molecular pre-treatment, implants of immortalized cells with dopaminergic phenotype, and finally dopaminergic cells proliferated and differentiated from ES, EG, or SS cells are the alternatives for cell supplementation therapy in PD. None of these approaches has as yet given a better answer to the PD problem. Some have reached phase I clinical trials; others are still in the animal laboratory research phase. However, all these approaches have raised new ethical questions, so that one cannot say that these alternatives have solved the ethical problems for groups in society that categorically condemn the use of any human embryonic or fetal cells for research or therapy. Each of these approaches has to deal with the aspects of tissue collection as well as with new safety aspects for the recipient patient of the clinical study.

Negative moral judgment about the use of human donor cells appears to be inversely related to the age of the human source. Ethical discussions on the use of in vitro human pre-implantation embryos as the source for ES cells provoked societal and political debate more strongly than at the time when the use of abortion remains was introduced as a possible therapeutic treatment for neurodegenerative diseases. The use of residual, otherwise destroyed IVF embryos must, however, be compared with the use of the tissue remains after elective abortion. The moral value of human life at the pre-implantation

stage of the blastocyst should not be placed above that of a post-implantation embryo in which organogenesis has started to create the appearance of a human body. In ethical evaluations of the human value of prenatal life the principle of growing relative protection with age is the generally accepted and main view. In addition, the use of ES cells is meant to develop cell lines for transplantation, so that, theoretically, large cohorts of patients may be treated with cells from a single abortion or a single surplus IVF embryo. The creation of human blastocysts, either from donated egg cells and sperm (e.g., IVF protocol) or from donated egg cells used for SCNT with the somatic nucleus of the patient (therapeutic cloning to obtain genetically identical ES cells to grow autologous transplants) must be regarded as using human life as a means-to-an-end for transplantation. Here one has to weigh the notion that in vitro human early life is not yet a "person," and the potential good for a person with a noncurable disease such as PD (or other life-threatening and severely handicapping diseases). At the present time, also in view of the recent findings that pluri- and multipotent SS cells might be isolated from adult organs as well as from umbilical cord blood, and may be possible to differentiate into virtually every cell type, a proof of principle for efficacious and safe transplantation with ES-derived neurons in PD should be presented before the need for creation of embryos has been decided upon. For cellular therapy in the nervous system it may not even be necessary to initiate therapeutic cloning to circumvent immune rejection, as the brain is an immunoprivileged site.

Besides methods to replace the lost dopaminergic input of the denervated striatum by cell placement, molecular interventions are also being developed, and clinical tests have started. Here, the safety aspects for, e.g., gene therapy in the CNS are to be considered. The brain is the organ of our mind, the complex network of our neurons make up our human behavior. Careful animal studies should always precede any clinical trial, taking into account possible side effects in functional capacity of the brain. Though neural grafting of embryonic mesencephalic tissue was found not to alter psychological and physiological measures to a great extent in PD subjects, any other putative but irreversible cellular or molecular treatment should reconsider these aspects. The field of restorative neurosurgery would be well served if the scientists involved in a clinical trial of any new approach were to publish the scientific and ethical justification of their program. This would allow discussion in the scientific and public domain, and help to take away the often publicly expressed distrust in "scientists playing God" with gene and human embryonic cell therapeutic means.

The publication of Freeman et al. (1999), which justified the double-blind sham-controlled study on the genuine efficacy of embryonic mesencephalic grafts in PD subjects is an excellent example of how discussion can be provoked and how it can lead to an open exchange of arguments on a sensitive topic: the human as research subject in irreversible experimental treatments. Freeman et al. (1999) stated that placebo effects and observer bias should be

eliminated in a meaningful study that includes patients. However, the gold standard of the double-blind placebo-controlled investigation model from the pharmacological field cannot be simply translated to restorative neurosurgery. Indeed, human experiments that gave non-interpretable results are unethical, but unnecessary control treatments in human beings are also. The more so because the risks of imitation surgery and further medical treatment and testing cannot be ignored, and informed consent is easily biased by therapeutic misconception for PD patients who have high hopes for any new therapy (Dekkers and Boer, 2001). These aspects may thus violate the basic ethical principles of beneficence, non-maleficence, and autonomy. The strict application of a CAP, including quantitative measures for the motor capacities, would very much help to avoid bias in the evaluation of the outcome of any type of restorative neurosurgery in PD. It can also render superfluous the need for the gold standard of double-blind placebo-controlled pharmaceutical studies in the experimental treatment of PD. Objective comparison between pre- and postsurgery measures is then possible as well as comparison between the new experimental therapies (as is done in clinical pharmacology when an old and a new drug are compared for their efficacy).

9. Acknowledgements

The author thanks Wilma Verweij for her text editing of the manuscript.

References

- Advisory Group on the Ethics of Transplantation (1996) *Animal tissues into humans*. Her Majesty's Stationary Office, Norwich, UK.
- Ahlskog, J.E., Muenter, M.D. (2001) Frequency of levodopa-related dyskinesias and motor fluctuations as estimated from the cumulative literature. *Mov Disord* 16:448–58.
- Albin, R.L. (2002) Sham surgery controls: intracerebral grafting of fetal tissue for Parkinson's disease and proposed criteria for use of sham surgery controls. *J Med Ethics* 28:322–5
- Arendash, G.W. and Gorski, R.A. (1982) Enhancement of sexual behavior in female rats by neonatal transplantation of brain tissue from males. *Science* 217:1276–1278.
- Arjona, V., Minguez-Castellanos, A., Montoro, R.J., Ortega, A., Escamilla, F., Toledo-Aral, J.J., et al. (2003) Autotransplantation of human carotid body cell aggregates for treatment of Parkinson's disease. *Neurosurgery* 53:321–8
- Armstrong, R.J. and Svendsen, C.N. (2000) Neural stem cells: from cell biology to cell replacement. *Cell Transplant* 9:139–52
- Bach, F.H., Fishman, J.A., Daniels, N., Proimos, J., Anderson, B., Carpenter, C.B., Forrow, L., Robson, S.C. and Fineberg, H.V. (1998) Uncertainty in xenotransplantation: individual benefit versus collective risk. *Nature Med* 4:141–144.
- Backlund, E.-O., Granber, P.-O., Hamberger, B., Sedvall, G., Seiger, A. and Olson, L. (1985) Transplantation of adrenal medullary tissue to striatum in parkinsonism: first clinical trials. *J Neurosurg* 62:169–173.

- Bankiewicz, K., Mandel, R.J. and Sofroniew, M.V. (1993) Trophism, transplantation, and animal models of Parkinson's disease. *Exp Neurol* 124:140–149.
- Barker, R.A., Kendall, A.L. and Widner, H. (2000) Neural tissue xenotransplantation: what is needed prior to clinical trials in Parkinson's disease? Neural Tissue Xenografting Project, *Cell Transplant* 9:235–46.
- Beauchamp, T.L. and Childress, J.E. (1989) *Principles of Biomedical Ethics*. New York: Oxford University Press, Oxford, UK.
- Benabid, A.L., Pollak, P., Gervason, C.L., Hoffmann, D., Gao, D.M., Hommel, M., et al. (1991) Long term suppression of tremor by chronic stimulation of the ventral intermediate nucleus thalamic nucleus. *Lancet* 337:403–6.
- Benabid, A.L. (2003) Deep brain stimulation for Parkinson's disease. *Curr Opin Neurobiol* 13:696–706
- Birnbacher, D. (1995) Identität der Persönlichkeit und Identität der Person: philosophische Fragen im Zusammenhang mit der Transplantation von Hirngewebe. *Z Neurochir* 5:180–185.
- Björklund, A. (1992) Dopaminergic transplants in experimental parkinsonism: cellular mechanisms of graft-induced functional recovery. *Current Opinion in Neurobiology* 2:683–689.
- Björklund, A., Dunnett, S.B., Brundin, P., Stoessl, A.J., Freed, C.R., Breeze, R.E., et al. (2003) Neural transplantation for the treatment of Parkinson's disease, *Lancet Neurol* 2:437–45.
- Bjornson, C.R., Rietze, R.L., Reynolds, B.A., Magli, M.C., Vescovi A.L. (1999) Turning brain into blood: a hematopoietic fate adopted by adult neural stem cells in vivo. *Science* 283:534–7.
- BMA guidelines on the use of fetal tissue. (1988) *Lancet* 1:1119.
- Boer, G.J. (1994) Ethical guidelines for the use of human embryonic or fetal tissue for experimental and clinical neurotransplantation and research. *J. Neurology* 242:1–13.
- Boer, G.J. (1996) The self-restraining ethical guidelines of NECTAR for the clinical neurotransplantation investigations. In: Hubig, C. and Poser, C. (eds.), *Cognitio humana—Dynamik des Wissens und der Werte*, XVII Deutscher Kongress für Philosophie, Leipzig; pp. 1420–7.
- Boer, G.J. (1999) Ethical issues in neurografting of human embryonic cells. *Theor Med Bioeth* 20:461–75.
- Boer, G.J. and Widner, H. (2002) Clinical neurotransplantation: core assessment protocol rather than sham surgery as control. *Brain Res Bull* 58:547–53.
- Bopp, J. and Burtchaell, J.T. (1988) *Report of the Human Fetal Tissue Research Panel, Vol 1*. Washington: US Government Printing Office, pp. 45–71.
- Borlongan, C.V. and Sanberg, P.R. (2002) Neural transplantation for treatment of Parkinson's disease. *DDT* 7:674–682
- Brazelton, T.R., Rossi, F.M., Keshet, G.I. and Blau, H.M. (2000) From marrow to brain: expression of neuronal phenotypes in adult mice. *Science* 290:1775–9.
- Brooks, D.J., Frey, K.A., Marek, K.L., Oakes, D., Paty, D., Prentice, R., Shults, C.W. and Stoessl, A.J. (2003) Assessment of neuroimaging techniques as biomarkers of the progression of Parkinson's disease. *Exp Neurol* 184 Suppl 1:S68–79
- Brundin, P., Karlsson, J., Emgård, M., Kaminski Schierle, G.S., Hansson, O., Petersen, A. and Castilho, R.F. (2000) Improving the survival of grafted dopaminergic neurons: a review over current approaches. *Cell Transplantation* 9:179–95.

- Butler, D. (1998) Last chance to stop and think on risks of xenotransplants. *Nature* 391:320–324.
- Calne, D.B. (1993) Treatment of Parkinson's disease, *N Engl J Med* 329:1021–7
- Cibelli, J.B., Kiessling, A.A., Cunniff, K., Richards, C., Lanza, R.P., West, M.D. (2001) Somatic cell nucleus transfer in humans: pronuclear and early embryonic development. *J Regen Med* 2:25–31
- Clark, P.A. (2002) Placebo surgery for Parkinson's disease: do the benefits outweigh the risks? *J Law Med Ethics* 30:58–68
- CNESVS (National Consultative Ethics Committee for Life Sciences and Health) (1984) *Recommendation on the Use of Embryo Tissue as well as Tissue of Dead Foetuses for Therapeutic, Diagnostic and Scientific Purposes*. Le Comité, Paris.
- CNESVS (National Consultative Ethics Committee for Life Sciences and Health) (1990) *Statement on Intracerebral Graft of Mesencephalic Tissue of Human Embryo Origin in Patients with Parkinsonism for Therapeutic Experimentation*. Le Comité, Paris.
- Council of Europe (1997) Convention for the protection of human rights and dignity of the human being with regard to the application of biology and medicine. *European Treaty Series* 164.
- CPMP Working Party on Efficacy of Medicinal Products (1990) EEC Note for Guidance: Good Clinical Practice for Trials on Medicinal Products in the European Community. *Pharmacol Toxicol* 67:361–72.
- Daar, A.S. (1998) Analysis of factors for the prediction of the response to xenotransplantation. *Ann NY Acad Sci* 862:222–233.
- Date, I., Shingo, T., Ohmoto, T. and Emerich, D.F. (1997) Long-term enhanced chro-maffin cell survival and behavioral recovery in hemiparkinsonian rats with co-grafted polymer-encapsulated human NGF-secreting cells. *Exp Neurol* 147:10–7.
- De La Fuente-Fernandez, R. and Stoessl, A.J. (2002) The biochemical bases for reward. Implications for the placebo effect. *Eval Health Prof* 25:387–98.
- De Wert, G. (2002) The use of human embryonic stem cells for research: an ethical evaluation, *Prog Brain Res* 138:405–470
- Deacon, T., Dinsmore, J., Costantini, L.C., Ratliff, J. and Isacson, O. (1998) Blastula-stage stem cells can differentiate into dopaminergic and serotonergic neurons after transplantation. *Exp Neurol* 149:28–41
- Defer, G.L., Widner, H., Marie, R.M., Remy, P., Levivier, M. and Conference partic-ipants (1999) Core assesement program for surgical interventional therapies in Parkinson's disease (CAPSIT-PD). *Movement Disorder* 14: 572–584.
- Dekkers, W. and Boer, G. (2001) Sham neurosurgery in patients with Parkinson's disease: is it morally acceptable? *J Med Ethics* 27:151–6.
- Donovan, P.J. and Gearhart, J. (2001) The end of the beginning for pluripotent stem cells. *Nature* 414:92–7.
- Dunnett, S.B. and Björklund, A. (1999) Prospects for new restorative and neuropro-protective treatments in Parkinson's disease. *Nature* 399:A32–39.
- Dunnett, S.B., Björklund, A. and Lindvall, O. (2001) Cell therapy in Parkinson's disease—stop or go?, *Nat Rev Neurosci* 2:365–9.
- Dunning, J.J., White, D.J. and Wallwork, J. (1994) The rationale for xenotransplanta-tion as a solution to the donor organ shortage. *Pathol Biol* 42:231–235.
- During, M.J., Kaplitt, M.G., Stern, M.B. and Eidelberg, D. (2001) Subthalamic GAD gene transfer in Parkinson disease patients who are candidates for deep brain stim-ulation. *Hum Gene Ther* 12:1589–91.

- During, M.J., Samulski R.J., Elsworth, J.D., Kaplitt, M.G., Leone, P., Xiao, X., Li, J., Freese, A., Taylor, J.R., Roth, R.H., Sladek, J.R.; O-Malley, K.L. and Redmond, D.E. (1998) In vivo expression of therapeutic human genes for dopamine production in the caudates of MPTP-treated monkeys using an AAV vector. *Gene Ther* 5:820–7.
- Dutch Health Council (2002) *Stem cells for tissue repair*, 09E, The Hague, The Netherlands
- EC Directive of the European Parliament and Council (2003) *Setting standards of quality and safety for the donation, procurement, testing, processing, storage and distribution of human tissues and cells*, 2002/0128 (COD), adopted A5-0387, Brussels, Belgium
- Falkner, F. and Tanner, J.M. (eds.) (1978) *Human Growth 1, Principles and Prenatal Growth*. Baillière Tindall, London, UK.
- Felten, D.L. (1994) Cell transplantation and research design. *Science* 263: 1546.
- Flax, J.D., Aurora, S., Yang, C., Simonin, C., Wills, A.M., Billingham, L.L., Jendoubi, M., Sidman, R.L., Wolfe, J.H., Kim, S.U. and Snyder, E.Y. (1998) Engraftable human neural stem cells respond to developmental cues, replace neurons and express foreign genes. *Nature Biotech* 16:1033–1039
- Freed, C.R., Greene, P.E., Breeze, R.E., Tsai, W.Y., DuMouchel, W., Kao, R., Dillon, S., Winfield, H., Culver, S., Trojanowski, J.Q., Eidelberg, D. and Fahn, S. (2001) Transplantation of embryonic dopamine neurons for severe Parkinson's disease. *N Engl J Med* 344:710–9.
- Freed, W.J. (1991) Substantia nigra grafts and Parkinson's disease: from animal experiments to human therapeutics trials. *Rest Neurol Neurosc* 3:109–134
- Freeman, T.B., Vawter, D.E., Leaverton, P.E., Godbold, J.H., Hauser, R.A., Goetz, C.G. and Olanow, C.W. (1999) Use of placebo surgery in controlled trials of a cellular-based therapy for Parkinson's disease. *N Engl J Med* 341:988–92
- Freese, A. (1999) Restorative gene therapy approaches to Parkinson's disease, *Med Clin North Am* 83:537–48
- Frodl, E.M., Duan, W.M., Sauer, H., Kupsch, A. and Brundin, P. (1994) Human embryonic dopamine neurons xenografted to the rat: effects of cryopreservation and varying regional source of donor cells on transplant survival, morphology and function. *Brain Res* 647:286–298.
- Gage, F.H., Björklund, A., Stenevi, U., Dunnett, S.B. and Kelly, P.A.T. (1984) Intra-hippocampal septal grafts ameliorate learning impairments in aged rats. *Science* 225:533–536.
- Galpern, W.R., Burns, L.H., Deacon, T.W., Dinsmore, J. and Isacson, O. (1996) Xenotransplantation of porcine fetal ventral meencephalon in a rat model of Parkinson's disease: functional recovery and graft morphology. *Exp Neurol* 140:1–13.
- Gansbacher, B. (2003) European Society of Gene Therapy. Report of a second serious adverse event in a clinical trial of gene therapy for X-linked severe combined immune deficiency (X-SCID). Position of the European Society of Gene Therapy (ESGT), *J. Gene Med* 5:261–2
- Gareth Jones, D. (1991) Fetal neural transplantation: placing the ethical debate within the context of society's use of human material. *Bioethics* 5:23–43.
- Gill, S.S., Patel, N.K., Hotton, G.R., O'Sullivan, K., McCarter, R., Bunnage, M., Brooks, D.J., Svendsen, C.N. and Heywood, P. (2003) Direct brain infusion of glial cell line-derived neurotrophic factor in Parkinson disease. *Nat Med* 9:589–95.

- Goetz, C.G., Janko, K., Blasucci, L., Jaglin, J.A. (2003) Impact of placebo assignment in clinical trials of Parkinson's disease. *Mov Disord* 18:1146–9.
- Hagell, P., Piccini, P., Björklund, A., Brundin, P., Rehncrona, S., Widner, H., Crabb, L., Pavese, N., Oertel, W.H., Quinn, N., Brooks, D.J. and Lindvall, O. (2002) Dyskinesias following neural transplantation in Parkinson's disease. *Nat Neurosci* 5:627–8.
- Hansen, J.E. (2002) Embryonic stem cell production through therapeutic cloning has fewer ethical problems than stem cell harvest from surplus IVF embryos. *J Med Ethics* 28:86–8.
- Harrower, T.P. and Barker, R.A. (2004) The emerging technologies of neural xenografting and stem cell transplantation for treating neurodegenerative disorders. *Drugs Today* 40:171–89.
- Heneine, W., Tibell, A., Switzer, W.M., Sandstrom, P., Rosales, G.V., Mathews, A., Korsgren, O., Chapman, L.E., Folks, T.M. and Groth, C.G. (1998) No evidence of infection with porcine endogenous retrovirus in recipients of porcine islet-cell xenografts. *Lancet* 352:695–9.
- HER (Working Group on Human Embryos and Research) (1994) *Second Report*, EC Directorate General XII, Science Research and Development, Brussels.
- Hermens, W.T. and Verhaagen, J. (1998) Viral vectors, tools for gene transfer in the nervous system. *Prog Neurobiol* 55:399–432.
- HMSO (Her Majesty's Stationary Office) (1989) *Review of the guidance on the research and use of fetuses and fetal material* (Polkinghorne rapport) London, Cm 762.
- Huffaker, T.K., Boss, B.D., Morgan, A.S., Neff, N.T., Strecker, R.E., Spence, M.S. and Miao, R. (1989) Xenografting of fetal pig ventral mesencephalon corrects motor asymmetry in the rat model of Parkinson's disease. *Exp Brain Res* 77:329–336.
- Hwang, W.S., Ryu, Y.J., Park, J.H., Park, E.S., Lee, E.G., Koo, J.M., Jeon, H.Y., Lee, B.C., Kang, S.K., Kim, S.J., Ahn, C., Hwang, J.H., Park, K.Y., Cibelli, J.B. and Moon, S.Y. (2004) Evidence of a pluripotent human embryonic stem cell line derived from a cloned blastocyst. *Science* 303:1669–74.
- Isacson, O., Deacon, T.W., Pakzaban, P., Galpern, W.R., Dinsmore, J. and Burns, L.H. (1995) Transplanted xenogeneic neural cells in neurodegenerative disease models exhibit remarkable axonal target specificity and distinct growth patterns of glial and axonal fibres. *Nature Med* 11:1189–1194.
- Isacson, O. and Breakefield, X.O. (1997) Benefits of hosting animal cells in the human brain. *Nature Med* 3:964–969.
- Itakura, T., Uematsu, Y., Nakao, N., Nakai, E. and Nakai, K. (1997) Transplantation of autologous sympathetic ganglion into the brain with Parkinson's disease. Long-term follow-up of 35 cases. *Stereotact Funct Neurosurg* 69:112–5.
- Kirik, D., Georgievska, B. and Björklund, A. (2004) Localized striatal delivery of GDNF as a treatment for Parkinson disease. *Nat Neurosci* 7:105–10.
- Koller, W.C. (ed.) (1987). *Handbook of Parkinson's Disease*. Dekker, New York.
- Kordower, J.H., Bloch, J., Ma, S.Y., Chu, Y., Palfi, S., Roitberg, B.Z., Emborg, M., Hantraye, P., Deglon, N. and Aebischer, P. (1999) Lentiviral gene transfer to the nonhuman primate brain. *Exp Neurol* 160:1–16.
- Kordower, J.H., Emborg, M.E., Bloch, J., Ma, S.Y., Chu, Y., Leventhal, L., et al. (2000) Neurodegeneration prevented by lentiviral vector delivery of GDNF in primate models of Parkinson's disease. *Science* 290:767–73.
- Langston, J.W., Widner, H., Goetz, C.G., Brooks, D., Fahn, S. and Freeman, T. (1992) Core assessment program for intracerebral transplantation (CAPIT). *Movement Disorders* 7:2–13.

- Larsson, L.C., Frielingsdorf, H., Mirza, B., Hansson, S.J., Anderson, P., Czech, K.A., Strandberg, M. and Widner, H. (2001) Porcine neural xenografts in rats and mice: donor tissue development and characteristics of rejection. *Exp Neurol* 172:100–14.
- Coffman, K.L., Sher, L., Hoffman, A., Rojter, S., Folk, P., Cramer, D.V., Vierling, J., Villamel, F., Podesta, L., Demetriou, A. and Makowka, L. (1998) Survey results of transplant patient's attitudes on xenografting. *Psychosomatics* 39:379–383.
- Le, H.N. and Frim, D.M. (2002) Gene therapy for Parkinson's disease. *Expert Opin Biol Ther* 2:151–61.
- Lindvall, O. (1999). Cerebral implantation in movement disorders: state of the art. *Movement Disorders* 14:201–5.
- Linke, D.B. (1993). *Hirnverpflanzung*. Rowoldt, Reinbek bei Hamburg.
- London, A.J. and Kadane, J.B. (2002) Placebos that harm: sham surgery controls in clinical trials, *Stat Meth Med Res* 11:413–427
- Macklin, R. (1999) The ethical problems with sham surgery in clinical research. *N Engl J Med* 341:992–6
- Madrazo, I., Drucker-Colin, R., Diaz, V., Martinez-Mata, J., Torres, C. and Becerril, J.J. (1987) Open microsurgical autograft of adrenal medulla to the right caudate nucleus in two patients with intractable Parkinson's disease. *New Engl J Med* 316:831–4.
- Martin, U., Kiessig, V., Blusch, J.H., Haverich, A., Von der Helm, K., Herden, T. and Steinhoff, G. (1998) Expression of pig endogenous retrovirus by primary porcine endothelial cells and infection of human cells. *Lancet* 352:692–694.
- Matthiesen, L. (2002) *Survey on opinions from national committees or similar bodies, public debate and national legislation in relation to human embryonic stem cell research and use, Vol 1–2*, EC Research Directorate-General, Brussels.
- McCullagh, P.M. (1987) *The Foetus as Transplant Donor: Scientific, Social and Ethical Perspectives*. Wiley, Chichester.
- McLaren, A. (1996) Research on embryos in vitro, the various types of research. *Third Symposium on Bioethics 'Medically assisted procreation and the protection of the human embryo'* CDBI/SPK 22, Council of Europe, Strasbourg December 15–18.
- McLaren, A. (2001) Ethical and social considerations of stem cell research. *Nature* 414: 129–31.
- McRae, C., Cherin, E., Diem, G., Vo, A.H., Ellgring, J.H., Russell, D., Fahn, S. and Freed, C. (2003) Does personality change as a result of fetal tissue transplantation in the brain? *J Neurol* 250:282–6.
- Mezey, E., Chandross, K.J., Harta, G., Maki, R.A. and McKercher, S.R. (2000) Turning blood into brain: cells bearing neuronal antigens generated in vivo from bone marrow. *Science* 290:1779–82.
- Nakao, N., Kakishita, K., Uematsu, Y., Yoshimasu, T., Bessho, T., Nakai, K., Naito, Y. and Itakura, T. (2001) Enhancement of the response to levodopa therapy after intrastriatal transplantation of autologous sympathetic neurons in patients with Parkinson disease. *J Neurosurg* 95:275–84.
- Nasto, B. (1997) Xenotransplant firms get xenophobic. *Nature Biotech* 15. Normile, D., Vogel, G., Couzin, J. (2006) South Korean team's remaning human stem cell claim demolished. *Science* 311:156–157.
- Normile, D., Vogel, G., Couzin, J. (2006) South Korean team's remaning human stem cell claim demolished. *Science* 311:156–157.
- Northoff, G. (1996) Do brain tissue transplants alter personal identity? Inadequacies of some "standard" arguments. *J Med Ethics* 22:174–180.
- Nuffield Council on Bioethics. (1996) *Animal-to-human transplants*. London, UK.
- Nuffield Council on Bioethics (2000) *Stem cell therapy: the ethical issues*. Dorchester, UK

- Oduncu, F.S. (2003) Stem cell research in Germany: ethics of healing vs. human dignity. *Med Health Care Philos* 6:5–16.
- O’Rahilly, R. and Müller, F. (1987) *Developmental Stages in Human Embryos*. Carnegie Institution of Washington, Washington.
- Ostrosky-Solis, F., Quintanar, L., Madrazo, I., Drucker-Colin, R., Franco-Bourland, R. and Leon-Meza, V. (1988) Neuropsychological effects of brain autograft of adrenal medullary tissue for the treatment of Parkinson’s disease. *Neurology* 38:1442–50.
- Outka, G. (2002) The ethics of human stem cell research. *Kennedy Inst Ethics J* 12:175–213.
- Palacios, R., Golunski, E. and Samaridis, J. (1995) In vitro generation of hematopoietic stem cells from an embryonic stem cell line. *Proc Natl Acad Sci USA* 92:7530–4.
- Patience, C., Takeuchi, Y. and Weiss, R.A. (1997) Infection of human cells by an endogenous retrovirus of pigs. *Nature Med* 3:282–286.
- Peel report, Department of Health and Social Security. (1972) *The Use of Fetuses and Fetal Material for Research, Report of the Advisory Group*. Her Majesty’s Stationary Office, London.
- Peschanski, M., Defer, G.L., Dethy, S., Hantraye, P.M., Levivier, M., Nguyen, J.P. and Cesaro, P. (1999) The need for phase III studies in experimental surgical treatments of Parkinson’s disease. *Adv Neurol* 80:651–3.
- Piccini, P., Brooks, D.J., Björklund, A., Gunn, R.N., Grasby, P.M., Rimoldi, O., et al. (1999) Dopaminergic release from nigral transplants visualized in vivo in a Parkinson’s patient. *Nature Neuroscience* 2:1137–40.
- Polgar, S., Morris, M.E., Reilly, S., Bilney, B. and Sanberg, P.R. (2003) Reconstructive neurosurgery for Parkinson’s disease: a systematic review and preliminary meta-analysis, *Brain Res Bull* 60:1–24
- Ralph, M.R., Foster, R.G., Davis, F.C. and Menaker, M. (1990) Transplanted suprachiasmatic nucleus determines circadian period. *Science* 247:975–978.
- Raymon, H.K., Thode, S. and Gage, F.H. (1997) Application of ex vivo gene therapy in the treatment of Parkinson’s disease. *Exp Neurol* 144:82–91
- Robertson, J.A. (1988) Rights, symbolism and public policy in fetal tissue transplants. *Hastings Center Report* 6.
- Rohwedel, J., Guan, K., Zuschratter, W., Jin, S., Ahnert-Hilger, G., Furst, D., Fassler, R. and Wobus, A.M. (1998) Loss of beta1 integrin function results in a retardation of myogenic, but an acceleration of neuronal, differentiation of embryonic stem cells in vitro. *Dev Biol* 201:167–84.
- Romijn, H. (1997) About the origin of consciousness. A new, multidisciplinary perspective on the relationship between brain and mind. *Proc Kon Akad Wetensch* 100:181–267.
- Sanberg, P.R., Borlongan, C.V., Othberg, A.I., Saporta, S., Freeman, T.B. and Cameron, D.F. (1997) Testis-derived Sertoli cells have a trophic effect on dopamine neurons and alleviate hemiparkinsonism in rats. *Nat Med* 3:1129–32
- Scothorne, R.J. (1968) Early development. In Passmore, R., and Robson, J.S. (eds.): *A Companion to Medical Studies*, Vol 1, Ch 18, Blackwell Scientific Publications, Oxford.
- Shamblott, M.J., Axelman, J., Littlefield, J.W., Blumenthal, P.D., Huggins, G.R., Cui, Y., Cheng, L. and Gearhart, J.D. (2001) Human embryonic germ cell derivatives express a broad range of developmentally distinct markers and proliferate extensively in vitro. *Proc Natl Acad Sci USA* 98:113–8.

- Shen, Y., Muramatsu, S.I., Ikeguchi, K., Fujimoto, K.I., Fan, D.S., Ogawa, M., et al. (2000) Triple transduction with adeno-associated virus vectors expressing tyrosine hydroxylase, aromatic-L-amino-acid decarboxylase, and GTP cyclohydrolase I for gene therapy of Parkinson's disease. *Hum. Gene Ther* 11:1509–19.
- Shetty, N., Friedman, J.H., Kieburtz, K., Marshall, F.J. and Oakes, D. (1999) The placebo response in Parkinson's disease. Parkinson Study Group. *Clin Neuropharmacol* 22:207–12.
- Singer, P. (1990) *Embryo Experimentation. Ethical, Legal and Social Issues*. Cambridge University Press, Cambridge.
- Stoye, J.P., Le Tissier, P., Takeuchi, Y., Patience, C. and Weiss, R.A. (1998) Endogenous retrovirus: a potential problem for xenotransplantation? *Ann NY Acad Sci* 862:67–74.
- Slevin, J.T., Gerhardt, G.A., Smith, C.D., Gash, D.M., Kryscio, R., Young, B. (2005) Improvement of bilateral motor functions in patients with Parkinson disease through the unilateral intraputaminial infusion of glial cell line-derived neurotrophic factor. *J Neurosurg* 102:216–222.
- Tasker, R.R., Siqueira, J., Hawrriylshyn, P. and Organ, L.W. (1983) What happened to VIM thalamotomy for Parkinson's disease. *Appl Neurophysiol* 46:68–83.
- Taylor, H., Minger, S.L., (2005) Regenerative medicine in Parkinson's disease: generation of mesencephalic cells from embryonic stem cells. *Curr Opin Biotechnol* 16:487–492.
- Tenenbaum, L., Lehtonenm, E. and Monahanm, P.E. (2003) Evaluation of risks related to the use of adeno-associated virus-based vectors. *Curr Gene Ther* 3:545–65.
- Thomson, J.A., Itskovitz-Eldor, J., Shapiro, S.S., Waknitz, M.A., Swiergiel, J.J., Marshall, V.S. et al. (1998) Embryonic stem cell lines derived from human blastocysts. *Science* 282:1145–1147.
- Toma, J.G., Akhavan, M., Fernandes, K.J., Barnabe-Heider, F., Sadikot, A., Kaplan, D.R., et al (2001) Isolation of multipotent adult stem cells from the dermis of mammalian skin, *Nat Cell Biol* 3:778–84.
- United Nations (1948) Universal declaration of human rights. *Yearbook of the United Nations 1948–49*. Dept of Public Information, United Nations, New York.
- Walters, L. (1988) Ethical issues in fetal research: a look back and a look forward. *Clin Res* 36:209–214.
- Webster, R.G., Guan, Y., Poon, L., Kraus, S., Webby, R., Govorkoval, E. et al (2005) The spread of the H5N1 bird flu epidemic in Asia in 2004. *Arch Virol Suppl* 19:117–129.
- Weiss, R.A. (1998) Transgenic pigs and virus adaptation. *Nature* 391:327–328.
- Weissman, I.L. (2002) Stem cells—scientific, medical, and political issues. *N Engl J Med* 346:1576–9.
- Widner, H. (1994) NIH neural transplantation funding. *Science* 263:737.
- Widner, H. and Defer, G.L. (1999) Dyskinesias Assessment: From CAPIT to CAP-SIT. *Movement Disorders* 14 Suppl 1:60–66.
- Wilmut, I., Schnieke, A.E., McWhir, J., Kind, A.J. and Campbell, K.H. (1997) Viable offspring derived from fetal and adult mammalian cells. *Nature* 385:810–813.
- Wolf, E., Zakhartchenko, V. and Brem, G. (1998) Nuclear transfer in mammals: recent developments and future perspectives. *J Biotechnol* 65:99–110.
- World Medical Association Declaration of Helsinki (2000) Ethical principles for medical research involving human subjects. *JAMA* 284:3043–5.
- Zurn, A.D., Tseng, J. and Aebischer, P. (1996) Treatment of Parkinson's disease. Symptomatic cell therapies: cells as biological minipumps. *Eur Neurol* 36:405–8.



<http://www.springer.com/978-0-387-29984-6>

Restorative Therapies in Parkinson's Disease

Brundin, P.; Olanow, C.W. (Eds.)

2006, XVII, 365 p., Hardcover

ISBN: 978-0-387-29984-6