

***INVITED REVIEW***

# Integrating fetal neural transplants into a therapeutic strategy: the example of Huntington's disease

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**Summary**

Fetal neural transplants have become clinically relevant over the past 15 years for two major neurodegenerative diseases, namely Parkinson's disease and Huntington's disease. It is therefore timely to consider how this neurosurgical procedure can integrate the therapeutic armamentarium, what can be expected of it, and what cannot. We use here the example of Huntington's disease to show what fetal neural transplants may uniquely offer for that disease. Up to very recent times, Huntington's disease has been one special example of those neurodegenerative diseases against which neurologists feel totally helpless. This has all changed today and, although results are essentially still to come, one can foresee the mobilization of very large scientific and

medical forces against this disease, with definite steps forward in terms of physiopathology and a better view of the therapeutic challenges. While defining the role that fetal neural transplantation may play in meeting these challenges, we also try to show rationales and developments for all types of treatments attempted or suggested so far, as well as their limits and, when relevant, informative failures. The date of writing this review needs to be noted, because the rapid accumulation of data on molecular mechanisms of Huntington's disease pathogenesis and the increasing numbers of clinical trials do not allow much time for the ink of a review to dry.

**Keywords:** neurodegenerative diseases; striatum; cell therapy; neuroprotection

**Abbreviations:** CNTF = ciliary neurotrophic factor

*Received October 15, 2003. Revised January 9, 2004. Accepted January 10, 2004.*

**Introduction**

Therapeutic research on Huntington's disease can be divided conceptually into two major trends that differ in concepts and methods, as well as (for the most part) scientific actors involved.

A first trend, which could be referred to as 'determinist', is characterized by attempts at elaborating treatments interfering with identified neuronal death pathways possibly triggered by the Huntington's disease mutation. In addition to strategies aiming at directly blocking the mutant gene [a therapeutic dream that will possibly come true in the foreseeable future with the introduction of RNA interference

(Tuschl *et al.*, 2002; Xia *et al.*, 2002)], this type of research is mainly based on the deciphering of the various intracellular cascades of cell death mechanisms that ultimately lead to neurodegeneration. In this context, essentially two main axes of research have been followed for some time, which are currently converging: the search for mechanisms of neuronal cell death in general and the identification of the deleterious role of the mutant Huntington's disease gene product itself.

A second, almost completely separate trend, which would be more adequately stamped as 'pragmatic', aims at

elaborating and evaluating potential treatments that take into account two general observations, namely that Huntington's disease provokes (at least in the early course of the disease) the preferential degeneration of medium-spiny GABAergic striatal neurons, and that this degeneration essentially evolves over a long period of time (neuronal degeneration comes after decades of apparent lack of major neuronal dysfunction). The first observation suggests that substitution of the lost striatal neurons (by cell replacement therapy) may offer some benefit. The second observation points to the existence of intrinsic neuronal defence systems which ought to be strengthened. In both cases, a precise knowledge of the cell death pathways involved in the disease process is not required to develop an effective treatment.

Nevertheless, when one considers elaborating a therapeutic strategy against the disease, and not only a therapeutic tool to oppose some of its effects, the distinction between these two main streams of research blurs as their complementarity is evident: reconstructing brain neural circuits is beyond the reach of any of them except fetal neural transplantation; conversely, protecting the brain against the progression of the disease is not a goal of such transplantation and will conspicuously be best obtained by interfering with the actual death pathways triggered by the disease. Neuroprotective and reconstructive therapeutics will, therefore, be unavoidably integrated into a common strategy, for which recent data indicate that fetal neural transplantation indeed represent a valid option.

## **Understanding Huntington's disease to prevent or stop the neurodegenerative process**

### ***Interfering with specific neuronal cell death pathways***

Mechanisms of neuronal death in Huntington's disease have been a major topic for neurobiological research for at least a quarter of a century (reviewed in Brouillet *et al.*, 1999). Notwithstanding the participation of the mutant protein itself in toxicity, which will be considered in the following section, several main concepts have been put forward successively, and secondarily integrated in composite schemes to rationalize Huntington's disease pathogenesis. These schemes still serve as working hypotheses on which current attempts at experimental pharmacological therapeutics are based.

### ***The excitotoxicity hypotheses***

The oldest concept, which arose in the 1970s, is that of excitotoxicity (now qualified as 'direct'). According to this hypothesis, glutamate (or more generally excitatory amino acids and structural glutamate agonist analogues) may trigger neuronal death by acting on specific receptors at the neuronal membrane. Excessive activation of these receptors, especially of the subclass that binds *N*-methyl-D-aspartate, opens calcium channels and allows massive influx of this ion into

the cells. This uncontrolled influx of calcium in turn activates a number of intracellular signalling pathways and enzymes, resulting in the so-called excitotoxic cascade of neuronal death (Olney *et al.*, 1971). As a consequence, excitotoxic lesions are characterized by a selective neuronal degeneration, with preservation of fibres of passage. In the striatum, administration of glutamate analogues can mimic the loss of the medium-spiny GABAergic neurons with preservation of interneurons, typical of Huntington's disease pathology (Coyle *et al.*, 1976; McGeer and McGeer, 1976; Beal *et al.*, 1986).

Blockade of cell death induced by direct excitotoxicity can be envisaged at two levels. Decreasing the presynaptic release of glutamate, and therefore reducing its local concentration at the neuronal membrane, may be achieved by acting on presynaptic systems that either link the arrival of action potentials with neurotransmission or participate in the reuptake of the neurotransmitter. Clinical trials based upon the use of riluzole or lamotrigine have been designed on this basis. Riluzole has demonstrated some neuroprotective effects in rats against excitotoxic striatal lesions. As concerns lamotrigine, a phase II study carried out in Canada has failed to demonstrate any significant benefit of the treatment (Kremer *et al.*, 1999). Decreasing the efficacy of the calcium channel associated with the *N*-methyl-D-aspartate receptors can also be attempted. Nevertheless, most of the molecules that block these receptors are not clinically relevant because of their intrinsic toxicity (e.g. MK 801). This explains why the Huntington Study Group has turned to remacemide, a partial blocker known to be innocuous, despite experimental results that indicated only transitory (Schilling *et al.*, 2001) and altogether weak effects of that drug against excitotoxic processes (Ferrante *et al.*, 2002). Results of a large-scale randomized controlled study, in which remacemide was compared with, and combined with, coenzyme Q10 (discussed below), have turned out negative (Huntington Study Group, 2001).

One potential reason for the lack of success with drugs that specifically interfere with direct excitotoxic processes may have been that this working hypothesis does not fully apply to Huntington's disease. Already in the 1980s a number of studies had failed to reveal any hyperactivity of the glutamatergic neurotransmission system *per se*. Henneberry and colleagues then introduced a major change in the excitotoxic hypothesis by showing that even under physiological concentrations of glutamate, a partial deficit in energetic metabolism could be sufficient to trigger the excitotoxic cascade in neurons (Novelli *et al.*, 1988). The major interest of this 'indirect excitotoxicity' hypothesis was that it not only provided a good rationale for the negative observations reported above but also integrated early observations of specific disturbances in energetic metabolism in the Huntington's disease striatum. Later studies additionally showed that prolonged energetic disturbance produced by chronic mitochondrial inhibition may, by itself, lead to lesions in the CNS that mimic those of Huntington's disease

(Beal *et al.*, 1995). According to the indirect excitotoxic hypothesis, a mitochondrial defect can therefore activate an excitotoxic cell death cascade, by provoking partial depolarization of the neuronal membrane and the abnormal activation of calcium channels associated with *N*-methyl-D-aspartate receptors.

Under such experimental conditions of mitochondrial inhibition, riluzole proved to be neuroprotective in rats against indirect excitotoxic striatal lesions induced by systemic administration of the mitochondrial complex II inhibitor 3-nitropropionic acid (Guyot *et al.*, 1997). This, however, was not replicated in non-human primates, in which the drug demonstrated an acute 'symptomatic' effect on choreic symptoms but no effect on dystonia (Palfi *et al.*, 1997). Indeed, this antichoreic effect of riluzole was subsequently observed in patients, in a pilot trial organized in Boston (Rosas *et al.*, 1999). A European clinical trial has since suggested that this motor benefit is only transitory (Seppi *et al.*, 2001). The neuroprotective efficacy of riluzole is currently under evaluation in a long-term, large-scale multicentre trial in Europe. Nevertheless, according to the indirect excitotoxicity hypothesis, mitochondria are now considered a second major target system for potential therapeutics for Huntington's disease. It has therefore been proposed to correct energy impairment by providing mitochondria with specific energetic substrates or by increasing the efficacy of the respiratory chain. Following these lines of research, creatine has shown some beneficial effects in animal models of Huntington's disease (Andreassen *et al.*, 2001), most likely by increasing the stock of phosphocreatine (Tarnopolsky and Beal, 2001). This compound is instrumental in keeping phosphate bonds at high energy, therefore facilitating rapid ATP formation in response to the cell's energetic demands. In parallel, strategies aiming at reducing the production of free oxygen radicals associated with the mitochondrial dysfunction, or of trapping them in cage compounds, have also been tested. Based upon such hypotheses, several molecules have proved beneficial in excitotoxic models, among which is the coenzyme Q10 (ubiquinone), an endogenous compound that permits efficient electron transfer in the respiratory chain. However, as already alluded to above in the paragraph on remacemide, coenzyme Q10 did not significantly alter the course of the disease in a large-scale study organized by the Huntington Study Group (Huntington Study Group, 2001). Despite claims of a 'trend toward improvement' (Greenamyre and Shoulson, 2002), the 13% difference observed between the group of patients treated by the compound alone and the placebo group did not reach statistical significance.

### **The apoptotic pathways**

During the 1990s, the deciphering of apoptosis pathways suggested a new group of potential therapeutic targets for Huntington's disease (Sanchez Mejia and Friedlander, 2001). However, this ever-increasing number of proteins and

systems with which therapeutic agents may interfere has not yet allowed clinically relevant drugs to be identified. Some of the potential therapeutic methods are being actively explored in animal models of Huntington's disease. In particular, this concerns approaches directly interfering with the initiation of the apoptotic cascade, for which several molecules have proved to be efficient *in vitro*. As an example, one may quote cyclosporin A, mostly known as an immunosuppressor drug but which may also prove to be neuroprotective as it blocks the opening of the mitochondrial transition pore, preventing the release of cytochrome *c* from the mitochondrial membrane into the cytoplasm, one of the initial steps of the apoptotic cascade. Unfortunately, like many other molecules, cyclosporin A does not readily cross the blood-brain barrier and it may be difficult to envisage long-term cotreatment with cyclosporin together with molecules which, like bradykinin, may temporarily alter it. Further down in the cascade, other molecules may also interfere with caspases. Overexpression of a dominant negative form of caspase 1 in transgenic mice expressing a mutant form of the first exon of the huntingtin gene (R6/2 mice) (Mangiarini *et al.*, 1996) slowed the disease process (Ona *et al.*, 1999). In addition to the synthetic peptides that are currently used experimentally, there may be room for the therapeutic use of endogenous caspase inhibitors like c-Flip, IAPs (inhibitors of apoptosis proteins), ICEBERG or Cop (for review see Onteniente *et al.*, 2003). Along the same lines, minocycline, a second-generation tetracycline which inhibits the production of nitric oxide by the inducible form of nitric oxide synthetase and inhibits downstream events of apoptosis, should be mentioned. Minocycline has demonstrated some neuroprotection in a mouse model of Huntington's disease and is currently under clinical investigation (reviewed by Friedlander, 2003).

### **Combating the effects of the huntingtin mutation**

Ten years ago, the identification of the mutant gene (Huntington's Disease Collaborative Research Group, 1993) opened another potentially fruitful path to therapeutics by allowing researchers to explore specifically the deadly mechanisms at work in the brain of patients carrying the Huntington's disease mutation. Although no potential treatments have been derived from these studies yet, there is an impressive number of lines of experimental research to identify causal mechanisms and strategies for treatment opened by this genetic identification. The mutated huntingtin protein itself appears capable of interfering in a major way with numerous intracellular systems (for recent reviews see Reddy *et al.*, 1999; Gutekunst *et al.*, 2000; Sipione and Cattaneo, 2001). Besides the multiple and complex relationships between caspases and huntingtin, one can underline three distinct, though not exclusive, pathological hypotheses that suggest potential therapeutic targets.

The first one points to direct alteration of cell functions by the mutated protein, in particular in the nucleus. This was strongly supported some years ago, when the first engineered mouse model of Huntington's disease (Mangiarini *et al.*, 1996) revealed nuclear inclusions (Davies *et al.*, 1997), which were subsequently identified in the brain of Huntington's disease patients (DiFiglia *et al.*, 1997). Although it has since been suggested that nuclear inclusions are not necessarily related to cell death (Saudou *et al.*, 1998), this hypothesis continues to be actively explored. First identified as pure aggregates of mutant huntingtin protein fragments, these inclusions have rapidly been shown to include a wealth of other proteins and protein complexes. These could be considered either as 'accomplices' or 'victims' of the mutation, depending upon the direct or indirect (through deprivation) toxicity resulting from their aggregation to huntingtin. Hen and colleagues have demonstrated that these inclusions are not stable in time, and that affected cells can get rid of them under some circumstances (Yamamoto *et al.*, 2000). The search for factors that could prevent the formation of those inclusions, or trigger their dislocation, may therefore be of therapeutic value. Chaperone proteins belong to those potential inhibitors (for review see Bonini, 2002) and cell biology experiments have shown that their activity may be directly stimulated *in vitro* (Sittler *et al.*, 2001), paving the way for *in vivo* trials.

A second research axis focuses on the ubiquitin–proteasome system (for review see Ciechanover and Brundin, 2003). This system of proteins, normally involved in the maturation of numerous proteins and in the degradation of abnormal ones, is rendered non-functional by aggregation with the mutant huntingtin (Bence *et al.*, 2001). The specific interest shown by many researchers in this partnership stems largely from studies on the proteasome (largely independently of its potential implication in Huntington's disease) revealing that its functional efficacy decreases with age (for review see Keller *et al.*, 2002). This characteristic may help solve one of the most vexing biological problems of Huntington's disease, namely its classical late onset in adulthood. This delayed onset—and the parallel long-term preservation of neuronal integrity and function in the striatum of patients—has been difficult to reconcile with hypotheses that point to the direct intervention of the mutant protein in cell death pathways. The functional ageing of the proteasome system offers a hypothetical explanation of this paradox, if one considers that degradation of abnormal proteins is sufficiently active for a long time in individuals, but is not permanent. Could the proteasome be a therapeutic target? Until recently, this seemed very unlikely since the fine tuning of this proteolytic system was apparently required to ensure cell survival. Nevertheless, new molecules have been designed that demonstrate modulatory effects in various pathological models, not only *in vitro* but also *in vivo* (e.g. Hosseini *et al.*, 2001). Therefore, hope exists for a potentially therapeutic stimulation of this system. Candidate molecules capable of fulfilling such a role remain to be identified.

The third research axis with potential therapeutic outcome that we believe should be emphasized derives from surprising results recently obtained by Cattaneo and colleagues that stress the need for a reconsideration of the widely accepted view that the mutation would only lead to a gain of function (Zuccato *et al.*, 2001). It was observed a long time ago that knock-out of the huntingtin gene in mice did not result in a disease process comparable to Huntington's disease but, rather, was lethal very early in development (Zeitlin *et al.*, 1995). Reciprocally, this suggested that the product of the mutant gene could keep sufficient functional activity to ensure survival, in particular considering that, though exhibiting slightly different symptoms (Squitieri *et al.*, 2003), homozygous patients are not strikingly more affected than patients heterozygous for the mutation. This view is now contradicted by the demonstration that the expression of the mutant protein decreases the expression of the native form which, in turn, strongly represses the promoter of a number of neuron-associated genes by freeing the gene suppressor REST (Zuccato *et al.*, 2003). Among those genes, and quite interestingly, is the one encoding the neurotrophic factor brain-derived neurotrophic factor. Accordingly, a decrease in brain-derived neurotrophic factor expression has been observed in the striatum of Huntington's disease patients, related to a decrease in expression in cortical neurons that mainly project to striatal target cells. Interestingly, brain-derived neurotrophic factor has been shown to protect neurons against the effects of the expression of the mutant protein in culture (Saudou *et al.*, 1998). These results strongly suggest, therefore, that striatal neurons in patients may benefit from the local administration of this missing physiological neurotrophic factor.

As a conclusion to this first part of the review, one may definitely acknowledge that this mechanism-based therapeutic research for Huntington's disease is flourishing. This is in sharp contrast to the current lack of positive results in the pharmacological trials that have been directly inspired by this line of research. As recently discussed by Rosser and Dunnett, (2002), it is also essential to keep the number of drugs assayed in Huntington's disease limited, given on the one hand the large number of patients that each of these trials requires and on the other hand the limited number of patients potentially recruitable. As a consequence, the choice of new potential therapeutic molecules has to be extremely careful. This may prove to be difficult even for the most expert teams, as the failures of the lamotrigine trial (Kremer *et al.*, 1999) and of the CARE–Huntington's disease multicentre trial of remacemide and coenzyme Q10 (Huntington Study Group, 2001) have emphasized. One way out of this dilemma has probably been paved by a recent initiative of the National Institutes of Health which has promoted a large-scale blinded characterization of the therapeutic potential of 1000 FDA-approved drugs on a number of genotypic cell and animal models of various diseases, including Huntington's disease. This study may be very helpful in sorting out the most clinically relevant drugs. It takes place, in fact, at the precise

border between the determinist line of research presented above (since it is based upon the genotypic models that are supposed to mimic the molecular basis of the pathology) and the pragmatic line of research presented below, since there is no attempt at deriving specifically a form of therapy from those mechanisms.

### **Halting Huntington's disease without understanding it: neurotrophic factors, gene transfer and neuroprotection**

A number of experimental studies, *in vitro* as well as in animal models of neurodegeneration, have demonstrated, without clearly revealing the mechanisms at work, that significant benefits could be obtained using diverse neurotrophic factors. Among these factors, ciliary neurotrophic factor (CNTF) deserves particular attention because it has been promoted recently to a first pilot trial evaluating the safety of its intracerebral administration in Huntington's disease patients, through a number of experimental studies in various models of the disease.

CNTF, which belongs to the interleukin 6 family of cytokines, was identified more than two decades ago as a trophic factor for parasympathetic neurons of the chick ciliary ganglion (Adler *et al.*, 1979). Since then, this acidic protein of 23 kDa and 200 amino acids has been shown to be widely distributed in the PNS, in Schwann cells and in the CNS, in which, though at lower concentrations, it is synthesized by astrocytes (Sendtner *et al.*, 1994). The full CNTF receptor is a heterotrimeric complex in which the dimeric LIF (leukaemia inhibitory factor) receptor is combined with an almost specific subunit (CNTFR  $\alpha$ ), which appears to be expressed only by skeletal muscle fibres and neurons in the CNS (MacLennan *et al.*, 1996). The physiological role of this protein has been elusive, due in particular to the absence of a signal peptide for the release by exocytosis. However, CNTF has been shown to stimulate the survival, proliferation or differentiation of a number of cell types *in vitro*. *In vivo*, it provokes the growth of neurites and reduces neuronal death during development as well as in diverse experimental conditions in the adult. Among various target cells that can be influenced by CNTF, it is interesting for our purpose to quote the striatal GABAergic neurons that appear to be rescued by this neurotrophic factor under different toxic situations (Anderson *et al.*, 1996; Emerich *et al.*, 1996, 1997; Mittoux *et al.*, 2000, 2002).

The analysis of pharmacokinetic parameters and of the toxicology of CNTF has revealed, however, a number of characteristics that preclude its systemic administration for the purpose of neuroprotection. Its plasma half-life has been evaluated to less than 3 min after intravenous injection, due essentially to major uptake by hepatocytes. In addition, the protein does not readily cross the blood-brain barrier and consequently does not reach a detectable concentration in the central parenchyma. Side effects, including inflammation

and cachexia, have also been recorded after systemic administration, and they have been severe enough to force the stopping of phase II/III clinical trials in patients with amyotrophic lateral sclerosis (Cedarbaum *et al.*, 1995; ALSCTSG, 1996; Miller *et al.*, 1996a, b).

These data have led to the investigation of delivery systems that allow continuous administration of CNTF directly into the brain of patients. Following a number of studies in rats and non-human primates that involved mechanical mini-pumps (Anderson *et al.*, 1996) or various types of gene therapy approaches (Emerich *et al.*, 1996, 1997; Mittoux *et al.*, 2000, 2002), we have chosen to use the macroencapsulation technique for a phase I trial (Bachoud-Lévi *et al.*, 2000c), in collaboration with Aebischer and colleagues in Lausanne (Switzerland). BHK cells engineered to synthesize and release large amounts of CNTF have been introduced into a tube formed by a semipermeable membrane, the pores of which are sized so that oxygen, nutrients and small proteins (like CNTF) can cross freely, whereas larger proteins (e.g. antibodies) and cells (and processes) cannot. One such capsule (diameter 0.6 mm, length 2.5 cm) was introduced into the lateral ventricle of six patients with Huntington's disease, using stereotaxic neurosurgery. The capsule was retrieved every 6 months and exchanged for a new one containing fresh cells. There was no major side effect recorded over the 2 year course of the study (four implantations) in the six patients, and clinical results will be presented shortly (J. Bloch, A.C. Bachoud-Lévi, N. Déglon, J.P. Lefaucheur, L. Winkel, S. Palfi, J.P. Nguyen, C. Bourdet, V. Gaura, P. Remy, P. Brugières, M-F. Boissé, S. Baudic, P. Cesaro, P. Hantrave, P. Aebischer and M. Peschanski, unpublished results). However, biological results (the amount of CNTF still being released by the capsule at retrieval) reveal that only in a minority of cases were significant amounts of CNTF still entering the CSF after 6 months in the Huntington's disease brain. Consequently, the phase II trial that was planned will only be organized after solving this biological issue of stable long-term CNTF delivery, most probably by modifying the type of encapsulated CNTF-producing cell.

### **Rebuilding the brain: the field of foetal neural transplantation**

The emergence of the general concept of neuroplasticity was, close to 20 years ago, at the origin of a suggestion for the treatment of Huntington's disease. According to this concept, neurons modify their own structures and connections throughout their life to address physiological needs. Neuroplasticity is now widely accepted, and implies that one can envisage the replacement of lost neurons in a circuit by providing afferent and efferent neurons with cells phenotypically similar to the lost ones. Accordingly, one may imagine treating Huntington's disease patients by introducing new cells into their striatum. This goal has been

actively pursued and there have recently been clinical trials which, though still within the framework of small-scale pilot studies, have been the first-ever attempt at a treatment for Huntington's disease to reveal some clinical efficacy.

Although the Huntington's disease pathology affects widespread areas in the brain early in the disease (Rosas *et al.*, 2003), the predominance of the striatal pathology in Huntington's disease, at least in the early stages of the disease process, is one main argument in favour of a substitutive therapy based upon the intrastratal transfer of homologous fetal neurons. However, the correlation between the striatal neurodegeneration and the clinical symptoms observed in patients has often been questioned and, although clinical benefits have been recently demonstrated in patients with striatal grafts (Bachoud-Lévi *et al.*, 2000b), the mere use of the technique is still denied by some authors on this basis (Greenamyre and Shoulson, 2002). Despite their large number and high level of agreement (for review see Peschanski *et al.*, 1995), it is therefore worth reassessing the data that clearly point to the primary and prolonged isolated involvement of the striatum in most Huntington's disease patients.

First, there is severe atrophy of both the putamen and the caudate (60% in common forms), and the link between the extent of this atrophy and the stage of clinical evolution of the disease is such that Vonsattel and colleagues (Vonsattel *et al.*, 1985) have graded the disease by reference to the degree of tissue loss in the striatum of the patients. The total functional capacity score, elaborated by Shoulson (Shoulson and Fahn, 1979) and commonly used to stage patients clinically, is closely linked to the anatomical grading of striatal atrophy previously defined by Vonsattel. More recently, imaging techniques based upon the analysis of either the cerebral blood flow or metabolic activity have largely, and consistently, confirmed the existence of a primary and major dysfunction of the striatum, well before other cerebral regions begin to show any alteration. Striatal hypometabolism, as assessed by PET, is profound (ranging from 37 to 91% of control values, according to studies and stages of the disease) and increases at a mean rate of about 7% per year (Kremer *et al.*, 1999).

The striatal substrate of the clinical symptoms in Huntington's disease patients has been supported repeatedly by correlative analyses combining imaging and neuropsychological data. In particular, Huntington's disease patients, like patients with Parkinson's disease, exhibit cognitive defects that plead strongly in favour of a subcortical origin, in sharp contrast with the symptoms related to the cortical pathology of patients such as those presenting with Alzheimer's disease. The triad aphasia–apraxia–agnosia does not exist in Huntington's disease patients, who rather demonstrate more diffuse symptoms marked by mnestic and executive defects, with attentional problems and slowness of mental processing. The progressive atrophy of the striatal nuclei seems to disconnect the prefrontal cortex (Lawrence *et al.*, 1998, 1999) and consequently patients display a frontal

syndrome, in the absence of an apparent primary defect in the prefrontal cortex. Interestingly, patients and non-human primates with pure striatal lesions display a similar pattern of frontal-type cognitive deficits.

The experimental basis of fetal neural grafting in various animal models of the disease, including mice, rats and non-human primates, impressively comprises hundreds of original papers (for reviews see Peschanski *et al.*, 1995; Kendall *et al.*, 1998; Fricker-Gates *et al.*, 2001) that strongly indicate that intrastratal implantation of striatal fetal neurons can reverse a large number of symptoms (both motor and cognitive) elicited by striatal lesions of various kinds. Such anatomical rebuilding of the neuronal circuits interrupted by striatal neurodegeneration, however, requires the adequate maturation of the grafted fetal neurons in the adult brain, the regeneration and outgrowth of host axonal afferents to the striatum reconnecting with grafted cells, and finally the directed outgrowth of axons from grafted neurons towards the appropriate host target cells and the formation of synaptic contacts. All experimental results in phenotypic animal models of Huntington's disease have given positive answers to these three prerequisites, forming a strong rationale for neural replacement in Huntington's disease.

Thus, several markers seem to indicate that fetal neurons do mature normally following intrastratal grafting, although the diversity of neuronal phenotypes in the striatum precludes a strict analysis of all neuronal populations. GABAergic neurons, which are the most prominent population in the striatum, appear also to be expressed in large portions of the grafts observed after transplantation of the fetal ganglionic eminences, especially when selective dissection of the lateral ganglionic eminence is used. These grafted neurons also contain various neuropeptides that are normally present in the striatum, such as substance P, met-enkephalin, somatostatin and neuropeptide Y, and display D1 and D2 dopaminergic receptors, as well as muscarinic receptors.

Concerning the re-establishment of functional connections between host afferent axons and the grafted cells, the picture is a little more complex (for review see Wictorin, 1992). Diffuse axonal systems, like those formed by monoaminergic afferents, readily grow into the grafts and connect grafted neurons in a quite specific way. In contrast, axonal regeneration and ingrowth into the graft are only partial for specific (so-called point-to-point) systems, such as those originating from the cerebral cortex or the thalamus. Innervation is then much denser in the periphery of the grafts, and only a few axons reach most central zones. Nevertheless, these anatomical axonal regenerations lead to the reconstitution of functionally efficient circuits, as assessed by electrophysiological recordings performed in acute striatal slices and *in vivo* (Xu *et al.*, 1991).

At the other end of the neuronal chain, a functional reconnection is possible between grafted cells and previously denervated target neurons. In rat models, grafted striatal neurons massively reinnervate the globus pallidus. In contrast only a few axons from allografted neurons reach more remote

projection zones, such as the substantia nigra, pars reticulata. Interestingly, however, the globus pallidus is by far the most important projection zone of striatal neurons in primates, including humans.

Behavioural analysis of grafted animals confirms the rewiring of cortical output circuits in which striatal neurons normally act as first relay cells. A striatal neuronal lesion provokes major motor defects in animals, as in humans, even though the complex array of motor symptoms (dyskinesias, dystonia and bradykinesia) observed in Huntington's disease patients is only mimicked in non-human primates (Hantraye *et al.*, 1990). With rare exceptions, the results of these studies demonstrate that striatal transplants elicit a clear beneficial effect in animal models of Huntington's disease. In rats, striatal grafts are able to reduce altogether the hyperactive behaviour provoked by a bilateral striatal lesion (Isacson *et al.*, 1984), as well as the rotations provoked by apomorphine or amphetamine following unilateral excitotoxic lesion (Dunnett *et al.*, 1988). Benefits have also been recorded when more complex tasks have been explored, such as the paw reaching test. In non-human primate models of Huntington's disease, bradykinesia and dyskinesias induced by unilateral excitotoxic lesions of the striatum can be reversed by intrastratal allograft of fetal striatal tissue (Kendall *et al.*, 1998).

The seriousness of the clinical impact of the disease is related, however, not only to motor but also to cognitive symptoms. This aspect has been difficult to comprehend in rodents, although pioneer studies (Isacson *et al.*, 1986) had already demonstrated that striatal allografts could contribute, though modestly, to the recovery of cognitive function assessed by a delayed alternating learning task. Mayer *et al.* (1991) and more recently Brasted *et al.* (1999) observed, using various cognitive tasks involving repeated learning sessions, that grafted rats recovered a level of learning efficiency that was close to normal values, whereas control lesioned-non-grafted animals never did. These results not only support the interpretation of the reconstruction by grafted neurons of an anatomical substrate necessary for these functions, but also the idea that, for functional recovery to occur in complex cognitive tasks, 'learning how to use the transplant' is a major prerequisite. We have produced a complementary demonstration of the cognitive function of striatal grafts by performing an analysis of a frontally related task in a macaque model of Huntington's disease (Palfi *et al.*, 1998). In this model, in which bilateral striatal neurodegeneration is progressively induced by daily systemic injections of 3-nitropropionic acid, an irreversible inhibitor of the mitochondrial enzyme succinate dehydrogenase, we have demonstrated full recovery of strategic adaptation (as assessed by the object retrieval detour task) over months following intrastratal bilateral allografting of fetal striatal tissue.

Five pilot clinical trials have been performed since the mid-1990s on the basis of these experimental data. Except for a recently published study carried out in Tampa, USA

(Hauser *et al.*, 2002), in which particularly advanced Huntington's disease patients were selected, studies concerned mostly patients in the early stage. Safety and feasibility of the grafting procedures appeared almost unquestioned (Kopyov *et al.*, 1998; Bachoud-Lévi *et al.*, 2000a; Fink *et al.*, 2000; Rosser *et al.*, 2002) except in the Tampa study, a result that may point to a particular sensitivity of advanced patients to neurosurgery and general anaesthesia. The one main concern shared by two of these groups (Bachoud-Lévi *et al.*, 2000a; Rosser *et al.*, 2002) related to immunosuppression, because of a risk of side effects. In Tampa, in contrast, three subjects developed subdural haemorrhages and two required surgical drainage (Hauser *et al.*, 2002). An autopsy performed by the same group (Freeman *et al.*, 2000), in one patient who died from a cause unrelated to the transplant 18 months after surgery, revealed the presence of well-developed grafts that contained large numbers of neurons phenotypically similar to GABAergic medium-spiny striatal neurons. One important result of that histological study was the demonstration that the grafted cells did not exhibit cellular marks of the disease, e.g. nuclear inclusions, whereas many of the host neurons in the surrounding striatum did so.

Conclusive clinical benefits have been shown so far only in the clinical trial performed in Créteil and Orsay, France (Bachoud-Lévi *et al.*, 2000b, 2002). The other study for which clinical data have been fully published (the Tampa trial) (Hauser *et al.*, 2002) was not conclusive, possibly because of the selection of particularly advanced patients, but also and most probably because of a too short follow-up time (12 months) (for commentary see Peschanski and Dunnett, 2002). In Créteil-Orsay, four patients out of the five who were transplanted demonstrated cognitive, motor and functional improvements. For one of them, this improvement was only transient, starting around 9–10 months after a first unilateral graft, and lasted up to 7 months, a few months after a second graft was performed on the opposite side of the brain. A systematic follow-up of this patient, which combined clinical assessment and brain imaging, revealed that the secondary loss of all improvements coincided precisely with the disappearance of striatal MRI hyposignals revealing the grafted tissue. This suggested the previous intrastratal development of grafted tissue (Bachoud-Lévi *et al.*, 2002), and confirmed the strict association between the presence of a graft and the clinical benefits. In the three other patients, motor, cognitive and functional benefits appeared about 12 months after the first graft and were still quite definite at the end-point chosen for the publication, 3 years after grafting (Bachoud-Lévi *et al.*, 2000b). A longer-term analysis, 6 years after grafting, will be presented shortly (A.C. Bachoud-Lévi, P. Rémy, P. Brugières, J.P. Lefaucheur, M.F. Boissé, S. Baudic, P. Maison, V. Gaura, C. Bourdet, P. Cesara, P. Hantraye, M. Peschanski, unpublished results).

Importantly, the clinical results recorded in Créteil-Orsay were matched by the evolution of the metabolic activity in the brain of those patients, as measured by PET using 18F-

fluorodeoxyglucose (Bachoud-Lévi *et al.*, 2000b). In non-grafted patients, metabolic activity declines by 7% annually (Kremer *et al.*, 1999). In the three patients who displayed clinical benefits, such decline was not observed; rather, increased <sup>18</sup>F-fluorodeoxyglucose consumption was observed in discrete areas of their grafted striatum, up to 60% above pregrafting values. Careful alignment of these regions of increased metabolic activity and MRI hyposignals observed at the site of implantation of the fetal tissue confirmed perfect coincidence. Interestingly, the subsequent analysis of the metabolic pattern in the whole brain of the same patients revealed that the intrastriatal grafting of fetal striatal tissue in these patients also induced recovery of metabolic function that was not only local but also involved large areas of the cerebral cortex, particularly the frontal lobe (Gaura *et al.*, 2004). Interestingly, the one patient who never experienced any benefit from the grafts had no increase in either striatal or cortical metabolic activity, but instead displayed a steady decline over time (Bachoud-Lévi *et al.*, 2002).

Substitutive therapy based on the intrastriatal implantation of fetal striatal tissue appears, at this time, to be the only experimental therapeutic that has demonstrated any long-term clinical benefit in some patients with Huntington's disease. So far, this therapeutic effect has only been observed in a very small population of patients, and in only one centre. The next step is the expansion of these experimental studies into much larger-scale, multicentre controlled trials (Peschanski and Dunnett, 2002). More than 100 Huntington's disease patients are currently involved in such trials in Europe, based upon the participation of multidisciplinary teams in the UK, France, Belgium, Germany, Switzerland, Italy and other countries. The precise value of fetal neural grafts as a means of therapy may, as a consequence, be fully evaluated in the coming 3–5 years.

## Conclusion

The present time is extremely rich in experimental research and clinical trials devoted to Huntington's disease. The different therapeutic approaches have for long been developed almost independently, most often by scientific teams that have rarely compared their views and data. The rapid development of clinical trials now imposes a change, since, as shown above, these different potential therapeutic approaches are in fact complementary and should be integrated within the framework of a common strategy against the disease. Symptomatic relief of chorea and psychiatric symptoms in particular seems within reach already, but should clearly be combined with a neuroprotective treatment as soon as one can be identified. Neural replacement, through fetal neural transplantation, which it is the only option at the present time, will be a welcome complement, providing patients with the possibility not only of stabilization but also even of the recovery of affected functions.

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