Cell therapy for Huntington's disease

Anne E Rosser
is a Clinical Scientist specialising in stem cell neurobiology, striatal development and cell therapy. As Professor of Clinical Neuroscience in Cardiff University she co-directs the Brain Repair group with SBD, and as Consultant Neurologist at the University Hospital of Wales she runs the specialist Huntington's disease research clinics in South Wales, including clinical trials of drug, rehabilitation and cell transplantation.

Stephen B Dunnett
is a Behavioural Neuroscientist specialising in animal models of basal ganglia disease and preclinical development of methods of cell transplantation and rehabilitation. He is a Professor in the School of Bioscience, Cardiff University, and co-directs the Brain Repair group with AER.

Correspondence to:
Prof Anne E Rosser,
Brain Repair Group,
School of Biosciences,
Cardiff University,
Museum Avenue,
Cardiff CF10 3AX, UK.

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Summary

- Early clinical trials of foetal striatal cell transplants in HD patients have shown initial indications of functional response, but the recovery has not (as yet) been shown to be reliable or sustained.
- In experimental animals, foetal striatal transplants can integrate into host circuitry and alleviate aspects of motor and cognitive disease, maintaining the prospect for an effective reconstructive cell therapy in HD patients.
- Cell transplantation can also be used for sustained and controlled delivery of neuroprotective and trophic molecules into precise deep brain targets, opening the prospect for alternative strategies to cell therapy which should be seen as complementary, not mutually exclusive.

What is meant by "cell therapy"?
Cells can be used therapeutically for two purposes: either to deliver substances to the brain, for example molecules that can support the survival of host neurons, or to replace cells that have been damaged or lost to the disease process with the aim of repairing the damaged neural circuitry (see Figure 1 for a schematic illustration of the principles of circuit repair versus substance delivery). The two purposes place different demands on the donor cells, and will be dealt with separately in this article. However, we emphasise that the different mechanisms of promoting recovery of function need not be mutually exclusive and may, at least theoretically, be combined into one more effective treatment strategy.

Why consider HD for cell therapy?
Huntington's disease (HD) is of interest as a target for cell therapy for two reasons:first because it is a devastating and currently untreatable disease with biological features that render it suitable for a cell therapy approach, and secondly because it is a good model of neurodegeneration more generally and may therefore allow the establishment of principles that can be generalised to other degenerative conditions.

Searching for treatments of HD
HD is said to affect around 6 per 100,000 in Europe, North America and Australia, although this may be a significant under-estimate of its prevalence.[1] Despite significant advances in the understanding of the pathophysiology and clinical phenotype of HD since discovery of the gene in 1993,[2] there is currently no available disease-modifying treatment for HD and symptomatic treatments are very limited and largely anecdotal rather than evidence-based.[3] While the molecular and cellular processes underlying HD are clarified and targeted, pharmacological treatments are sought and trialled, it is logical to pursue all rational strategies, which currently include empirical screening of existing drug libraries; therapies that potentially target the pathophysiology such as histone deacetylase inhibitors; RNA inhibition and similar strategies that developed from the understanding that HD is largely due to a toxic gain of function of the mutant protein; and replacement of cells based on the understanding that medium spiny neuron (MSN) damage plays an important role in the evolution of symptoms.[4]

Why is HD a suitable target for cell replacement?
HD presents biological features that makes it a good cell therapy target. In particular, the cell loss in HD, at least in the early to moderate stages of manifest disease, is predominantly of the medium spiny neurons (MSNs) of the striatum,[5] which normally constitute approximately 85% of the neurons in the intact human striatum. Thus, there is a focal area of degeneration and a single cell type to provide a target for cell placement. Although it is theoretically possible that cell therapy will eventually be suitable for diseases with diffuse degeneration extending over widespread and or involving multiple cell types, at this stage of evolution of the technology cell transplantation has been more successful when based on targeting replacement of a single or restricted range of cell types with a focal location amenable to direct surgical targeting. Of course, both the specificity of the cells injected and the "focal" nature of the disease are both relative rather than absolute constraints, and each is considered further below.

Another reason for investigating HD as a clinical target for cell replacement is that it presents a valuable model of neurodegeneration more generally in which to work out how to achieve success in cell therapy. There are compelling reasons for considering regenerative medicine in a wide range of neurodegenerative conditions. Together, these conditions represent a very large disease burden and, for the vast majority, there is no disease-modifying treatment currently available. Targeted pharmacological treatments are likely to be a long way off for most of these conditions, in which the detailed pathogenesis is not fully elucidated, and yet many of them are amenable to cell replacement because their anatomy and distribution of neuronal cell loss is understood.

There are several reasons why HD is a good model in which to understand the principle of

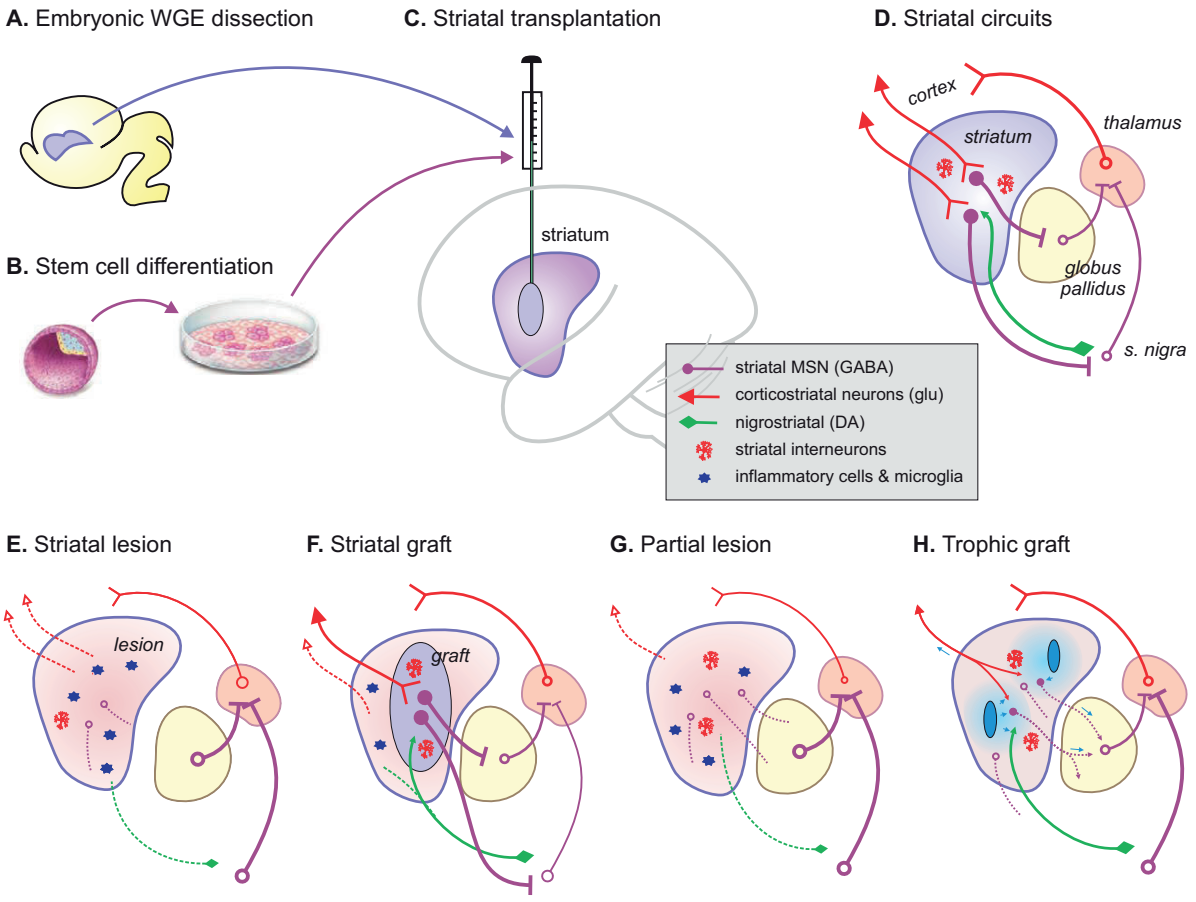

Figure 1: Schematic illustration of transplantation of primary embryonic ganglionic eminence (A) or stem cell-derived striatal neurons (B) into the host striatum (C), illustrating potential mechanisms of action:normal striatal connections relay information from cortex via intrinsic striatal processes to pallidum, thalamus and midbrain (D). Excitotoxic lesions destroy the striatal medium spiny projection neurons, accompanied by inflammatory and glial responses, yet with relative sparing of interneurons and host afferent terminals (E,G). Secretory grafts may provide a source for diffuse or locally regulated release of neuroprotective, anti-inflammatory and trophic factors, which enhance host neuronal survival, axon growth and plasticity, but do not replace essential circuit neurons destroyed by the lesion (H). By contrast, some grafts (such as fetal WGE) can replace lost striatal neurons leading to reconstruction of host neuronal circuits and recovery of function through true circuit repair (F). It remains undetermined by which mechanism the modest functional effects reported following stem cell-derived neuronal transplants are achieved.

cell repair. First, it is an autosomal dominant condition with full penetrance and a simple genetic test is available, which means that it can be diagnosed with certainty in life and indeed, prior to symptom manifestation.[6] This substantially increases the power of clinical trials aimed at disease modification, specifically, seeking to alter the course and progression in HD. Secondly, following the two decades after the discovery of the gene there has been substantial advancement of understanding of the underlying pathophysiology[7] and the clinical phenotype[8], underpinning a significant amount of work to optimise clinical outcome measures (for example reference 9), and building a platform for clinical trials.[8] Thirdly, there are multiple animal models (rodent, primate and model organisms), which provide an excellent laboratory platform for discovery and preclinical work-up of novel therapeutics. All of this means that HD is well set up for studies of novel therapeutic strategies.

The donor cells:circuit repair versus secreted molecules

The major difference between donor cells for molecule delivery and those for circuit reconstruction is that the former need to be capable of sustained secretion of the target molecules, but do not necessarily need to differentiate into a specific neural phenotype (see Figure 1A-D, G, H), whereas the latter must be capable of differentiating precisely to the cell type that are lost in the disease process, and then integrating appropriately into the host circuitry following transplantation (see Figure 1A, C-F).

Some cells such as mesenchymal stem cells appear to naturally secrete trophic-like molecules, although they may also be genetically engineered to produce specific molecules such as BDNF,[10] but they do not necessarily need to differentiate into neural cells themselves. There is some evidence starting to emerge suggesting that molecule delivery may be appropriate for HD. For example, mesenchymal stem cells engineered to produce BDNF (which is reduced in the brain in HD) appear to improve symptoms in animal models of HD and a clinical trial is now ongoing.[11] Available evidence suggests that the functional effects in this case are not due to structural repair whereby the exogenous graft cells replace those lost in the disease, but rather to the grafts acting as a vector for delivery of trophic and tropic stimuli to promote regenerative plasticity and endogenous reorganisation within the damaged host circuits. In the interest of space, this will not be considered further here and the focus will be circuit repair. Although presenting more stringent requirements on the cells, this strategy has a greater potential to generate improvements in function through structural repair of the core pathology. Our goal is true "brain repair", i.e. reversing the disruption of host circuits by replacement of lost neurons and authentic reconstruction of the damaged brain networks, thereby allowing restitution of the neural processing required to underlie normal complex motor and cognitive function.

In order for donor cells to be able to replace those lost to the disease process, they need to differentiate very precisely into the appropriate phenotype; to the extent that cell replacement is specific, cells that have some, but not all, of the features of the target cell may not demonstrate effective repair and functional improvement.[12] A good example of this is replacement of nigrostriatal dopamine neurons in Parkinson's disease: the target cells are A9 group of dopamine cells in the midbrain, and transplant studies have shown

that the adjacent A10 dopamine neurons are considered less capable of generating full repair and functional recovery;[12] indeed, other dopaminergic neurons of hypothalamic or olfactory origin do not show comparable integration in the host brain and are without functional impact on even simple motor features associated with nigrostriatal degeneration. The same appears to be true for replacement of pure populations of MSNs in HD. Other non-striatal GABAergic neurons are relatively ineffective in striatal lesion animals, and indeed the better functional results are achieved when the full population of striatal neuronal types – interneurons as well as MSN projection neurons – are included into the grafts.

Furthermore, in order to integrate into the host neuropil, donor cells must be immature and not fully differentiated, but at the same time must be committed developmentally to a specific phenotype so that once transplanted they are able to continue their differentiation pathway in a cell-autonomous fashion. This balancing act is crucial; a cell that is too immature will not have received all the developmental signals it needs to instruct it to become a specific neural subtype, so it may arrest at an immature developmental stage or follow a 'default' differentiation pathway. A cell that has completed differentiation and undergone significant maturation may not survive the transplantation process and quickly loses the early potential for rapid growth, neurite extension, connecting to appropriate targets and integration into the host neuronal network. In practical terms this means that there is a "developmental window" during which cells can be successfully transplanted for circuit reconstruction, corresponding roughly to the peak in embryonic birth dating of the target neuronal population. For human embryonic striatal cells for use in HD, this translates to approximately week 8-10 foetal tissue.

Evidence that circuit repair can work

Transplantation of developing primary foetal MSNs (i.e. MSNs obtained directly from the fetal striatum without manipulation in culture, as distinct from stem cell-derived neurons differentiated to MSN fate) into the degenerating striatum has been shown to ameliorate motor and cognitive deficits in animal studies, primarily in rats and primates. Such studies have allowed the mechanisms underlying the functional improvement to be explored, and have shown that implanted cells can integrate into the circuitry and make functional synaptic connections, providing that they were of the appropriate phenotype (i.e. destined to become MSNs) and were procured within the appropriate developmental window.[13] Evidence of functional efficacy in humans comes from a seminal French study that reported human fetal-derived graft survival and significant improvements in both motor and cognitive function in three patients.[14] Enhanced FDG-positron emission tomography signal in the frontal cortex of these individuals suggested that the implanted cells had integrated into the striatal neural circuitry and made functional connections with relevant cortical regions.[15] The improved cognitive function is particularly interesting, as there have been few treatments for cognitive impairment across any neurodegenerative disease. Clearly, further evidence is required with greater patient numbers; indeed, the French HD network has recently completed a larger transplantation study, which will hopefully be reported within the coming year. Overall, it is reasonable at this stage to conclude that there is proof-of-concept evidence that transplantation of developing MSNs into the striatum can produce functional improvements in at least some patients with HD. The task now is to improve reliability and to identify which cells, patients, and conditions provide the optimal functional response.

Challenges and the way forward

A major challenge for the field is that primary foetal cells are scarce and cannot be easily standardised, so a renewable, quality-assured source of cells is required. Various stem cell sources can be readily expanded in number, easily cryopreserved and are much more amenable to processing according to good manufacturing practice (GMP) principles. There are several human stem cell sources being actively explored for potential cell replacement therapy in the central nervous system, including embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), foetal neural precursors (FNPs), adult neural stem cells, and somatic stem cells derived from blood, bone marrow or other peripheral lineages.[16] The most critical factor in producing neurons with the capacity to repair the damaged adult brain is that they must reliably and accurately replicate the phenotype of those cells lost to the disease process. A small number of groups have demonstrated that neurons with MSN characteristics can be differentiated from human stem cell populations with survival of MSN-like cells post-transplantation.[17-20] Successful generation of MSN-like neurons has been achieved by exposing ESC-derived neuronal precursors to developmental signals thought to be important in MSN differentiation and a number of published protocols report differentiation of MSN-like cells in vitro and following transplantation into a rodent model of HD, where they provided variable functional improvement with some evidence that the cells could integrate into the host neural circuitry to receive dopaminergic input from the midbrain and glutamatergic input from the cortex while projecting fibres to the globus pallidus.[17-20] Several of these studies have also reported modest functional effects,[18,19] although it remains as yet undetermined the extent to which MSN-like stem-cell derived neurons have the same capacity to reconstruct the damaged host neural circuitry to a comparable degree to that readily achieved by authentic developing MSNs (see Figure 1). It is important to note that these cells are not currently ready for clinical translation, although that remains a topic of active investigation[21] and progress to date indicates that the current barriers to translation are surmountable.

A second challenge will be the fact that, although the focus of degeneration in HD is the striatum, there is also degeneration of extrastriatal regions. In the French studies outlined above, function continued to improve over the first few years, but patients started to decline again by six years post-surgery, most likely due to continued degeneration of the striatum.[22] While the improvement was not permanent, there are a number of reasons why transplantation should still be considered as a therapeutic option. First, this scale of improvement is substantially greater than any other attempted treatment of HD to date. Second, whereas the initial focus and mechanism of the spread of HD pathology within the diseased brain remains unresolved, it remains plausible that replacement of lost neurons in a critical node such as the striatum may provide additional support to afferent neurons and reduce prion-like transmission of toxic products,[23] thereby slowing the cell-to-cell spread of pathology within the neural circuitry. Third, cell replacement therapy is still at an early experimental stage and, judging by history such as renal transplantation in the 1960s where rather limited early success ultimately led to great medical advances, it is highly likely that optimising technical aspects and parameters (such as transplantation earlier in the disease) will produce more sustained effects. Finally, it would seem logical to ultimately consider cell replacement therapy in combination with disease modifying drugs once they also become available.

Transplantation as a therapeutic approach in Huntington's disease is at an early but exciting stage. Experimental models suggest that a surgical replacement strategy is feasible; multiple cell sources are available; multiple potential mechanisms of integration and functional recovery remain plausible. In the opinion of these authors, all options should remain open for theoretical and experimental exploration, without prejudice or pre-supposition, and dogmatic declarations of the 'correct' approach remain premature.

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