Transplantation in HD: Are We Transplanting the Right Cells?

Sophie V. Precious and Claire M. Kelly

Abstract

Cell replacement therapy is a viable option for the treatment of Huntington’s disease (HD), where the aim is to replace the lost medium spiny projection neurons of the striatum. The intra-striatal engraftment of developing striatal precursors harvested from the foetal brain has provided proof of concept in both rodent models and human patients that these primary foetal tissue grafts can bring about a degree of functional recovery in a HD-degenerated brain. With the advent of pluripotent stem cell technologies, novel, potential alternative donor cell sources have become available. Ongoing studies are assessing the capacity of these cells to differentiate towards striatal precursors for transplantation in HD. Here, we review the characteristics of potential donor cells for HD with respect to available cell markers, functional properties and maturity of cells upon transplantation. We consider the optimal composition of the donor cell population, that is, whether a heterogeneous population containing all cell types from the developing striatum (the whole ganglionic eminence) is preferable to a more homogeneous population of striatal projection neurons, as directed by differentiation protocols applied to pluripotent stem cells. Furthermore, we consider what might be required to improve transplant efficacy and success, with respect to striatal differentiation of transplanted cells and functional improvement.

Keywords: neural transplantation, primary foetal tissue, pluripotent stem cells, striatal medium spiny neurons, DARPP-32

1. Introduction

The application of cell transplantation as a therapeutic for the neurodegenerative disease Huntington’s disease (HD) offers to replace those striatal cells lost to the disease. Medium-sized spiny projection neurons (MSNs) of the striatum are the predominant cell type lost, and
It is these cells which we endeavour to replace in order to initiate reconstruction of the damaged circuitry and alleviate some of the symptoms associated with the disease. This specific and focal loss of MSNs in HD makes it an ideal candidate for cell replacement therapy.

Cells from the developing striatum (named the whole ganglionic eminence (WGE); the striatal primordia), harvested during the window of striatal neurogenesis and implanted into the HD brain, have shown beneficial effects with a degree of functional recovery in preclinical rodent studies and in ‘proof-of-principle’ clinical trials, see [1–3]. This indicates that intra-striatal transplantation of developing MSNs has the potential to alleviate some aspects of this disease. All clinical investigative studies of transplantation in HD have, to date, utilised human primary foetal tissue as the donor tissue [2, 3], where developing striata are harvested from multiple embryos obtained after elective termination of pregnancy. This donor tissue source has many limitations associated with it, leading to the ongoing quest to find an alternative cell source that can fulfil the requirements for successful transplantation, integration and functional improvements.

In this chapter, we will discuss the use of human primary foetal tissue, and what we know to date with respect to intra-striatal transplantation of this donor tissue source in the HD paradigm. The unanswered questions related to this donor source will be assessed, including what the optimal parameters might be for transplantation. We will consider the need for alternative donor cell sources and will look at the characteristics of potential alternative donor cell sources, and in particular, their ability to generate striatal MSNs \textit{in vitro} and post-transplantation and the factors potentially influencing their ability to improve function following transplantation.

2. Primary foetal tissue

It is well documented that the gold standard donor cell source for neural transplantation in HD is primary foetal tissue [4], where cells are taken from the developing brain from the region of origin of the desired mature cells and within an appropriate gestational window. Striatal MSNs originate in the WGE, which is situated within the developing telencephalon, and can be harvested easily using microdissection techniques [5, 6]. This can be straightforward depending on the method of tissue collection (i.e. medical versus surgical termination of pregnancy: MTOP and STOP, respectively), CNS tissue being more accurately dissected from MTOP-derived tissue than STOP-derived tissue due to less fragmentation of MTOP tissue, thereby enabling easier identification of different regions [7]. Thus, as a source of donor cells for transplantation, there has been a progressive move to the use of MTOP rather than the much more limited supply of STOP tissue. This in part reduces, albeit to a small extent, some of the logistical burden associated with the use of foetal tissue for cell replacement therapy. However, there are unknowns and limitations associated with the use of primary foetal tissue, which will be discussed in detail later.

Initial studies of cell transplantation in HD have provided accumulative evidence of the conditions for safety and preliminary evidence for clinical efficacy. There have been seven small clinical transplantation studies reported to date, all of which have used primary foetal striatal
tissue as the donor cell source [8–14]. Safety and feasibility of bilateral intra-striatal transplantation in HD patients have been shown [8–11].

Utilising magnetic resonance imaging (MRI), the trial based in California, USA [8], indicated graft survival in all three of their transplanted patients at 1-year post-transplantation. A separate trial, based in Florida, USA, reported a decrease in the Unified Huntington’s disease rating scale (UHDRS) score at 12-months post-transplantation, suggesting an improvement in motor function [11]. The INSERM trial, conducted in Créteil in France, provided more solid efficacy data [15]. Out of five patients with HD who received bilateral striatal implants of foetal WGE tissue, three had surviving grafts as evidenced by changes in MRI signal and increased metabolic activity in the graft regions on 18F-FDG positron emission tomography (PET). The three patients with surviving grafts were reported to show substantial motor and cognitive improvements at 1-year post-transplantation, as assessed using the Core Assessment Program for Intracerebral Transplantation in Huntington’s Disease (CAPIT-HD) [15]. At 6-years post-transplantation, again using the CAPIT battery, the over-riding message from this trial was the stability of the disease progression; in particular, the choreatic movements experienced by the patients remained stable, at an improved level for 4–6 years [16]. Further reports employing imaging techniques following striatal transplantation in HD have shown metabolically active tissues [14] and increased striatal D2 receptor binding with PET [12]. In addition, data from the Florence cohort of eight patients showed a degree of stabilisation or improvements in some neurological indices over 18- to 34-months post-transplantation [14], whilst the London cohort revealed some clinical improvement over 5-years post-transplantation [12].

The longest clinical follow-up assessment post-transplantation reported comes from the Cardiff-Cambridge, UK trial [17]. Data are presented for clinical outcome measures up to 10-year post-transplantation. They report a ‘trend towards a slowing of progression’, and although there were improvements found on certain measures for individual patients, there were no overall statistically significant improvements found in CAPIT scores between grafted patients and a non-grafted reference group. However, data obtained from PET imaging showed no obvious surviving graft tissue, and the authors postulate that the grafts were insufficiently large to produce a clinical benefit. Overall, the aforementioned trials have suggested that intra-striatal grafting is feasible and largely safe; disease progression has not been reported to accelerate in transplanted patients [10], and for patients showing no indication of graft benefits, progression of the disease appears similar to that seen in non-grafted patients [16]. These studies have also shown that human foetal striatal transplants can survive long term and can bring about functional benefits to symptomatic HD patients in at least some cases. What is less clear currently is what factors are important for producing graft-related benefit in a more reliable fashion. Potential contributing factors that need to be considered for successful primary foetal striatal transplants include gestational age of donor tissue, tissue dissection, tissue preparation, number of cells transplanted and selection of graft recipient, among others, reviewed in [18].

In addition to the clinical data discussed above, there are a number of published reports of post-mortem analyses from these transplant trials [13, 19–23]. The earliest post-mortem time was 6-month post-transplantation [13], and in this study, the authors reported graft-derived
DARPP-32 (dopamine- and cyclic-AMP-regulated phosphoprotein of molecular weight 32 kDa), NeuN, calretinin, somatostatin and GFAP, as well as graft innervation of host-derived tyrosine hydroxylase fibres. Markers of more immature precursors were also present, including doublecortin, Sox2 and Ki67 [13]. One patient from the Florida cohort died 18-month post-transplantation due to cardiovascular disease, and post-mortem analysis showed surviving graft tissue, which was positive for striatal markers such as acetylcholinesterase (AChE), calbindin and calretinin, as well as innervating tyrosine hydroxylase-positive processes [21]. Moreover, there was no evidence of immune rejection in the graft region or evidence that the graft was affected by the underlying disease progression [21]. Analysis of a graft from the California cohort also showed no signs of rejection or evidence of HD-related pathology in the graft [20]. However, this latter study, reporting on one patient at 10-year post-transplantation, revealed the presence of multiple mass lesions and cysts, suggestive of graft overgrowth. Additionally, although calretinin, calbindin, parvalbumin and neurofilament markers were reported, only rare neuronal projections traversing the graft-host boundary were noted [20]. One post-mortem study from the Florida trial, 10-year post-transplantation, demonstrated graft survival with expression of markers of striatal projection neurons and interneurons and evidence of synaptic connections between transplanted neurons and host-derived dopaminergic and glutamatergic neurons, but also suggested some degeneration of grafted neurons [22]. A further post-mortem analysis from the Florida cohort, up to 12-year post-transplantation, observed that there were both fewer blood vessels and fewer astrocytes in the graft compared with the surrounding host tissue, which together may result in reduced trophic support to the graft and impact on graft survival [23]. However, these grafts also showed some typical striatal graft morphology in which there were regions of the grafts that were positive for AChE, termed p-zones, as well as areas with no expression of AChE, termed non-p-zones [23].

Overall, it can be said that the data obtained from these limited numbers of transplant trials are somewhat mixed, in terms of both clinical outcomes and post-mortem analyses. It is difficult to draw any direct comparisons between data from the various studies because of the differences between studies in the protocols for tissue dissection, preparation, transplantation, immunisation and patient assessments, thus highlighting the need to undertake better controlled studies with common protocols to allow comparison of results between centres, reviewed in [3, 18].

3. What do we still need to know about human foetal WGE in order to improve graft reliability?

Although both animal research and clinical research into foetal striatal transplantation for HD span over two decades, several important issues relating to the optimal conditions for use of this tissue as a donor source of cells remain. The success of neural transplantation depends on harvesting the foetal tissue from the appropriate part of the developing CNS, at the appropriate gestational age, and for the preparation to be optimised to maximise cell viability.

The first unknown is how to optimise the dissection of the developing foetal striatum. During development, the striatum forms as two ridges in the floor of the embryonic lateral ventricles:
the lateral and medial ganglionic eminences (LGE and MGE, respectively). DARPP-32-positive MSNs derive predominantly from the LGE [24], whilst striatal interneurons are predominantly derived from the MGE [25]. Based on this, it has previously been proposed that deriving donor cells from the LGE, rather than WGE, would generate a purer population of MSNs and that this would produce an improved graft [26–29]. However, studies of rodent-to-rodent grafts show similar behavioural improvement in both LGE- and WGE-derived grafts, although the overall striatal graft volumes and mean numbers of striatal-like neurons were greater in the WGE-derived grafts. Thus, contrary to expectation, it is suggested that the presence of interneurons from the MGE may facilitate graft survival and integration, thus favouring a WGE-derived cell population for transplantation [29–31]. Studies of human foetal brain samples show DARPP-32-positive MSNs beginning to appear in the LGE from 7-weeks post-conception with the number increasing over the following 2 weeks [32, 33], but to date there have been no systematic studies using human foetal donor tissue in animal models to address the issue of ‘optimal dissection’, largely due to the scarcity of tissue.

It is known that the foetal gestational age is important in deriving donor cells that will go on to produce a functional graft, but a second unknown is the optimal foetal donor age for this purpose. In rodent studies, it has been shown that grafts derived from embryonic day (E) 14–E16 rat donors generate a higher proportion of striatal-like tissue compared with grafts derived from older embryonic tissue [30]. However, functional recovery was only seen in those recipients who received transplants from the younger E14 donors [34]. This has not been systematically investigated to date in any one, single study for human foetal samples. Thus, it is necessary to draw what we can from the published literature in which a range of ages from 6- to 14-week gestation has been used [35–38]. It has been shown that human foetal WGE cells harvested at 7- to 9-week post-conception [37] and also at 14-week post-conception [36] are able to ameliorate the apomorphine-induced deficits seen in animals having received unilateral excitotoxic striatal lesions, which mimic the pattern of cell loss seen in HD. In agreement with these earlier studies, we too see an improvement in apomorphine-induced rotations using human foetal WGE at 8-week post-conception [38]. Furthermore, improvement was also seen in the vibrissae-evoked forepaw placing test, as well as stabilisation over time in the adjusting steps test [38]. Together these studies build on the histological assessments of cell survival and integration. As described above, clinical trials have utilised tissue in the range of 6- to 12-week gestation, making this a potentially significant source of variation. Thus, despite the logistical difficulties (largely due to the uncertainties of foetal tissue availability) of undertaking comparisons of different gestational ages of human foetal WGE human to rat grafts, this is clearly a critical factor that needs to be extensively and systematically addressed.

A third factor to be considered is the way in which the foetal donor tissue is prepared prior to transplantation. Two broad approaches have been used to date: the crude chopping of the tissue into smaller pieces [8, 9, 11] and the mechanical dissociation of the tissue with the aid of enzymes [10, 12, 14]. As with the previous issues, there is again limited systematic evidence supporting either method. One study that examined this issue directly (using rodent tissue) reported a greater proportion of striatal-like tissue in conjunction with more DARPP-32 immunopositive neurons within grafts derived from dissociated cell suspensions compared
to grafts derived from tissue fragments [39]. Conversely, a modest improvement in functional recovery on the paw-reaching test was seen in animals receiving tissue fragment grafts compared to suspension grafts [39]. With current legislation pertaining to good manufacturing practice (GMP), there is a need to replicate this study using non–animal-derived products and so replacing the classical trypsin approach with GMP-compatible products.

In light of what has been discussed above, it is clear that there are many unknowns when it comes to the transplantation of human foetal striatal tissue in HD. It is critical that the questions raised above are not dismissed as new cell sources are investigated, and one key step in preclinical validation of these alternatives will be to compare them to primary foetal tissue transplants. One important consideration relates to the functional readout from such studies, especially given the limited data thus far generated from transplants of human foetal tissue [35–38]; some rodent studies have highlighted the plasticity of striatal grafts, which can have implications on functional effects post-transplantation [40–44]. It has been shown that animals post-transplantation need to ‘re-learn’ a task that had been well established prior to induction of the lesion, using the 9-hole box operant chamber [40]. Here, it was reported that simply reforming the circuitry in the brain was not enough to achieve functional benefit, but instead the animals needed a period of time to re-train in order to make use of the reconstructed circuitry. A similar strategy using a different task, the paw-reaching/staircase test, also showed the benefits of additional training post-transplantation [41].

Another important consideration which might enhance functional recovery in human foetal striatal transplant studies is the role for environmental enrichment which has been shown to favourably affect the behavioural readout in rodent allograft experiments [45]. Housing animals in an enriched environment post-transplantation resulted in larger projection neurons with increased spine density and better graft re-innervation [45, 46]. In addition, levels of BDNF in the intact side of the brain were increased in both transplanted and non-transplanted animals that were exposed to environmental enrichment compared with those in standard housing [43, 45, 46]. Furthermore, the impact of the enriched environment on the plasticity of striatal grafts has also been shown electrophysiologically, by measurement of long-term potentiation (LTP), which indicates persistence of synaptic strength. LTP was more readily induced in the grafts where hosts had received enrichment compared with those where hosts were in standard conditions [44, 47, 48].

In the studies of human foetal tissue transplants in animal models described above, the behavioural effects reported have been limited to drug-induced rotations, vibrissae-evoked touch test and adjusting steps test, and so far no effect has been reported on the paw-reaching test [35–38]. However, to date, neither the approach of additional training to allow transplanted animals to re-learn a task post-transplantation, and learn to use the graft, nor the environmental enrichment strategy has been applied to human foetal striatal transplant studies. One limitation of behavioural analysis in xenotransplantation studies is the restricted time window post-transplantation due to the need for daily immunosuppression. Despite the presence of DARPP-32-positive cells in the brains of these animals upon post-mortem analysis, transplanted human foetal striatal cells might require a longer time in vivo in order to achieve a functional readout as seen in the equivalent rodent studies. One way of overcom-
ing this would be to use the ‘neonatal desensitisation’ approach, which negates the need for daily immunosuppression [49]. However, this too has its own limitations not least the lack of understanding behind its mechanism of action.

4. Are foetal donor cells a long-term prospect?

There are a number of advantages associated with the use of human foetal striatal donor cells. The prime advantage is, as discussed above, the generation of MSNs that have been exposed to patterning signals during natural development and are thus likely to be ‘authentic’ MSNs with the greatest ability to bring about functional improvement in HD models and HD patients. However, there are additional advantages associated with the lineage-restricted nature of these cells, in particular, that there is a reduced risk of non-neural cells arising from the graft, and thus a much reduced risk of graft overgrowth and/or teratoma formation. These factors are why, currently, human foetal WGE cells are the ‘gold standard’ with which newer donor sources need to be compared. However, the continued use of human WGE cells in both animal and human studies extends beyond the simple comparison of efficacy. Given the uncertainties of the current clinical studies outlined above, there is a need for further proof-of-concept studies and to gain further insight into factors important for graft optimisation, including not only considerations of the donor cells, but also factors such as optimum host age and stage of disease. Moreover, understanding in more detail how foetal cells survive and integrate will be crucial in learning how to generate effective cells from other starting sources such as human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells.

Nevertheless, although it is important to continue to study human foetal WGE for the reasons above, it is unlikely that they will be sufficient to achieve widespread clinical application due to several ethical and logistical issues. First, the scarcity of this tissue supply is limiting. This is complicated by the requirement to use multiple donors per patient; some studies have used up to eight foetal donors per patient for a bilateral transplant, albeit that many studies have used 1–3, see [3]. It is also further limited by the need to harvest cells at the point of peak MSN neurogenesis, believed to be in the range of 8- to 12-week post-conception, thus further reducing the number of suitable, potential donor tissue retrievals. Moreover, the shift in working practices at gynaecological units means that the STOP tissue source is becoming even more rare and the MTOP tissue, due to the very nature of the procedure, can in some cases be completed in the comfort of the person’s own home, thus limiting the supply being procured through hospital facilities. A second issue is that following dissection, the tissue cannot be stored for long periods of time (maximum of 8 days) [50, 51]. Therefore, coordinating the tissue collection and transplantation can be logistically challenging. The organisational network that needs to be in place in order for clinical transplants to take place is exceptionally complex, in particular, the coordination of timing of foetal tissue collection (which it is not possible to manoeuvre), with the neurosurgical procedure. Another point to be mentioned (related to the inability of this tissue to be hibernated) is that the cells cannot be subject to full screening, tissue typing, etc, as they can’t be stored for long enough to complete such assessments, prior to transplantation. These considerable limitations associated with the use of primary foetal
tissue have led to the search for possible alternative donor cell sources to permit more widespread and better controlled transplant processes for the future.

5. Alternative donor cell sources

Desirable characteristics of donor cells to replace foetal WGE cells include: (i) the potential to proliferate in vitro whilst ensuring stability of the quality; (ii) the capacity to be expanded in vitro so as to generate large numbers of cells to overcome the issue of tissue supply; (iii) the ability to be stored, ideally cryopreserved, so that batches of cells with the same quality may be generated and frozen for subsequent use; (iv) having the capability to be responsive to inductive developmental cues (i.e. exogenous factors) in order to generate the target cellular phenotypes for neural transplantation (i.e. for HD, striatal MSNs); and (v) following transplantation, being able to repair the circuitry damaged in the disease process and bring about functional recovery.

Together, these desirable traits would sidestep the issue of tissue supply and the quality control caveats that come with the use of primary foetal tissue, as well as standardisation of cells for implantation. They would also circumvent the logistical hurdles with respect to retrieval of tissue for dissection and preparation, and coordinating with neurosurgical teams for implantation procedures. However, it is paramount that any alternative donor cell source be able to achieve the goal of generating the specific, authentic mature phenotype following transplantation and then differentiation and maturation in vivo in the adult striatum.

The catalogue of alternative donor cell sources for potential use in cell replacement strategies for neurodegenerative diseases is predominantly comprised of expandable cells that may be derived from embryonic, foetal or adult tissues and may be pluripotent, multipotent or theoretically, even unipotent.

Foetal neural precursors (FNPs) are multipotent cells, which are already restricted to a neural lineage, see [52]. Specifically, striatal FNPs are derived from foetal WGE, can be expanded in vitro to increase cell numbers and because of their origin of derivation may have the potential to differentiate more readily to a striatal phenotype. In terms of therapeutic application, the rationale is to expand the WGE-derived cells in vitro to increase the total cell population and be able to perform one, or more, complete, bilateral transplants per foetal donor.

However, assessment of human FNPs expanded in vitro has revealed differential gene expression between relatively early and late time points, 4–8 weeks and 20 weeks, respectively; in particular, revealing differences in genes is a key for providing information on positional identity, whilst expression of the neural markers, Nestin and Sox2, remains stable [53, 54]. Furthermore, there is a negative correlation between length of time in expansion culture conditions and yield of neurons upon subsequent differentiation; that is, with increased time in vitro, FNPs have a propensity to yield fewer neurons [55]. In addition to this, post-transplantation, human striatal FNPs that have been expanded in culture and undergone passaging produce fewer surviving grafts, with reduced neuronal differentiation and a lower yield of striatal neurons [53, 54, 56, 57]. Thus, it appears that in vitro expansion of striatal FNPs, at
least long term, limits the differentiation potential of these cells, which could be due to a loss in positional identity over time in culture. This, in turn, would mean that when long-term expanded cells are placed in an environment such as the adult brain and are not exposed to the developmental signals that they would see in the developing brain, they are unable to differentiate into phenotypes appropriate to the site from which they were derived (e.g. MSNs from striatally derived FNP).

On the other hand, short-term expanded striatal FNP's maintained in culture for 10 days, without passaging, yielded 41% neurons, 70% of which were immunopositive for the striatal MSN marker DARPP-32 (unpublished observations). Further to this, we have previously compared survival and axonal outgrowth of transplants of human primary foetal striatal tissue with short-term expanded (10 days) striatal FNP's, where we found richer cellular outgrowth from the FNP-derived grafts [58]. Recently, we have reported that striatal-derived FNP's expanded for short periods in culture prior to transplantation yield the same number of DARPP-32-positive neurons in grafts as those derived from primary foetal WGE [59]. Furthermore, we provided evidence to suggest that short-term expanded (2 and 9 days) striatal FNP's can bring about a degree of functional recovery, specifically on the corridor task (testing bias towards the ipsilateral side and neglect of the side contralateral to the lesion and transplant), following transplantation into an HD rat model [59]. Collectively, this indicates that FNP's, as a potential donor cell source for application in clinical transplantation, should not be overlooked, but should be further investigated to establish their true potential.

Pluripotent stem cells (PSC's) include ES cells and iPS cells, which have the capacity to generate any cell of the three germ layers: mesoderm, endoderm and ectoderm. Mouse ES cells were first identified in 1981 [60, 61], and more recently, human ES cells were also derived [62] from the inner cell mass of the blastocyst. iPS cells, derived from adult somatic tissues, were first generated in 2006, when mouse fibroblasts were re-programmed using retrovirus-mediated transfection and the transcription factors Oct3/4, Sox2, c-Myc and Klf4 [63]. Later, human iPS cells were generated from human adult fibroblasts using the same four factors [64]. This seminal paper on derivation of human iPS cells showed that these cells are similar to human ES cells with respect to proliferation capacity, pluripotency, gene expression, morphology and telomerase activity [64]. The last decade has been fruitful in the publication of research looking at pursuing PSC's (both ES and iPS cells) as potential donor cell sources for clinical application. It is imperative to remember that whatever the donor source, the cells need to be directed to a striatal MSN phenotype.

6. Directed differentiation of PSCs towards striatal MSNs

With a focus on human-directed differentiation studies of PSCs, we will discuss the development of protocols utilised in attempts to achieve striatal MSN phenotypes in vitro and in vivo. The key identifier used in these studies for confirmation of MSN production is DARPP-32, which is expressed by more than 95% of striatal MSNs. However, it is important to note that DARPP-32 is only expressed in mature MSNs and not precursor cells. Thus, when developing protocols for the generation of such cells, particularly from PSC sources, it is vital to have...
other markers available in order to determine that the differentiation progression is appropriate to the desired lineage and phenotype. Two such candidate markers, FoxP1 and CTIP2, have been identified as important markers, both of which label MSN precursor cells and co-label with mature DARPP-32 immunopositive MSNs [65, 66].

Initial studies that aimed to generate striatal MSNs applied factors to influence the neural induction of ES cells and downregulate the pluripotent and proliferative traits of these cells. Neural lineage induction of human ES cells was achieved using a variety of methods, including culture on feeder cells prior to adherence on substrate for further differentiation, feeder cell-conditioned medium and feeder-free suspension culture [67–70]. Human ES cells cultured using defined neural induction medium in free-floating suspension generated cells expressing markers of immature neural precursors such as Sox1 and Pax6 [70, 71]. Terminal differentiation of these precursor cells yielded β-III-tubulin immunopositive neurons that expressed GABA (gamma amino butyric acid; the principal neurotransmitter of striatal MSNs) after 70 days in culture [71].

More recently, a highly robust method of enhancing neural conversion of human ES cells has been developed utilising SMAD signalling inhibitors [72]. Specifically, addition of both noggin and SB431542 (a BMP inhibitor and Activin/Nodal inhibitor, respectively) was shown to increase the yield of cells expressing the neural markers Pax6, Foxg1 and Sox1, whilst expression of the pluripotent marker Oct4 decreased [72]. This method is now widely used as the first stage in the generation of neural cells from PSCs and has successfully been applied to the initial stages of striatal differentiation protocols [73–75] (discussed below, and see Table 1).

The process of striatal neuron generation from PSCs requires exposing the cells to various inductive stages and ‘patterning’ them so they may obtain the desired identities, by introducing signalling molecules indicative of regionalisation and specification, appropriate to the striatum. Following neural lineage induction, the cells need to be directed towards a striatal precursor lineage and then differentiated to generate the specific cell fate, that is, mature striatal MSNs.

Table 1 highlights studies that have reported protocols for differentiation of PSCs towards striatal neuron phenotypes, with analysis of both cultured cells in vitro and transplanted cells in vivo. The earliest report describing successful yield of DARPP-32-positive neurons from human ES cells used the mouse stromal feeder-cell method to generate neural rosettes, which were then directed towards striatal precursors and then terminally differentiated to neurons, a protocol that required more than 62 days [68]. The growth factors used in this study during the striatal patterning phase included SHH (sonic hedgehog), DKK-1 (Dickkopf) and BDNF (brain-derived neurotrophic factor), followed by dbcAMP (dibutyryl cyclic AMP), VPA (valproic acid) and BDNF for the terminal differentiation. Efficient generation of striatal MSNs in vitro was described, with cells expressing the striatal neuronal markers DARPP-32, GABA, calbindin and calretinin. Of the 22% of total cells that were MAP2+ neurons, 53% expressed DARPP-32. Although analysis of these cells following intra-striatal implantation into a rat excitotoxic model of HD revealed DARPP-32-positive neurons within the graft region (21% of total neurons), transplants contained cells expressing markers of persistent proliferation and showed teratoma-like overgrowth [68], thus raising issues about the efficacy of this protocol.
### Table 1. Studies reporting *in vitro* and *in vivo* analysis of striatal differentiation protocols with human pluripotent stem cells.

| Study | Cell lines | Protocol | DARPP-32+ neurons *in vitro* Day of analysis/observations | DARPP-32+ neurons *in vivo* Day of transplantation/observations |
|-------|------------|----------|----------------------------------------------------------|---------------------------------------------------------------|
| Aubry et al. [68] | Human ES cells: SA-01 & H9 | Neural induction in serum-free and N2-supplemented medium for 21–23 days; striatal patterning with addition of BDNF, SHH & DKK-1 (days 46–59); neuronal differentiation with dbcAMP, VPA & BDNF (continued > day 62) | Between days 62–72: 22% total cells were MAP2+ neurons, 53% neurons were DARPP-32+ | Day 59: DARPP-32+ cells at 4–6 weeks and 13–21 weeks post-transplantation; DARPP32+cells made up 21% neurons at 13 weeks, with no difference at later time points |
| Ma et al. [76] | Human ES cells: H9 | Neural induction in serum-free and N2-supplemented medium for 10–12 days; striatal patterning with SHH or purmorphamine (to day 26), then VPA (to day 32); neuronal differentiation with BDNF, GDNF, IGF, AA &cAMP (to day 47) | Day 47: 93% total cells were β-III-tubulin+ neurons, 90% neurons were GABA+, ~90% GABA+ were DARPP-32+ | Day 40: DARPP-32+ cells at 4 months post-transplantation; DARPP-32+/GABA+ neurons were 58% total graft-derived cells |
| Delli-Carri et al. [73] | Human ES cells: H9 & HS401 Human iPS cells: DF3F & WT iPS 3F-1 | Neural induction in serum-free medium with increasing concentration of N2-supplementation and addition of dorsomorphin, noggin, SB431542; addition of SHH & DKK-1; neuronal differentiation with N2, B27 & BDNF | Day 45: 80% of cells were β-III-tubulin+ neurons, majority were GABA+ some of which were DARPP-32+. Day 80: 51% total cells were Map2ab+ neurons, 20% neurons were DARPP-32+ | Day 38: DARPP-32+ cells at 9 weeks post-transplantation were 0.05% of total graft-derived cells |
| Nicoleau et al. [74] | Human ES cells: H9 & RC9 Human iPS cells: i90c17 | Neural induction with LDN (or noggin) & SB431542 for 10 days; further 10 days in N2/B27 medium; neuronal differentiation with BDNF, dbcAMP & VPA; (also tested addition of SHH or cyclopamine, and Wnt3a, DKK-1 or XAV-939) | Day 20 differentiated for a further 25 days: DARPP-32+ neurons present (optimal with 1μm XAV) Longer term for >60 days: DARPP-32+ neurons, with 23 fold more expression at day 60 than day 10 | Day 25: DARPP-32+ neurons extensive throughout grafts 5 months post-transplantation |
| Arber et al. [75] | Human ES cells: H1 & H7 Human iPS cells: 2F8 & 4FH | Neural induction in N2/B2 medium with SB431542 (up to day 5), LDN (or noggin) & dorsomorphin (up to day 9); addition of Activin A from day 9 (to day 20); terminal differentiation with BDNF & GDNF | Day 36–40: DARPP-32+ neurons, QPCR,5 fold increase with Activin treatment than without; ICC,20–50% DARPP-32+ (depending on cell line) | Day 20: DARPP-32+ cells 8 weeks post-transplantation (very few); 16 weeks post-transplantation, 49% HuNu+ cells were DARPP-32+ |

**Abbreviations:** BDNF, brain-derived neurotrophic factor; SHH, Sonic Hedgehog; DKK-1, Dickkopf; dbcAMP, dibutyryl cyclic AMP; VPA, valproic acid; GDNF, glial-derived neurotrophic factor; IGF, insulin growth factor; AA, ascorbic acid; FGF, fibroblast growth factor; XAV-939, chemical antagonist of Wnt/β-catenin pathway (substitute for DKK-1) (Wnt inhibitor molecule); QPCR, semi-quantitative real-time polymerase chain reaction analysis; ICC, immunocytochemistry; HuNu, human nuclei; DARPP-32, dopamine- and cyclic-AMP-regulated phosphoprotein of molecular weight 32 kDa; GABA, gamma amino butyric acid.
and highlighting the importance of in vivo verification post in vitro differentiation. A shortened neural induction period for generation of neuroepithelial cells followed by addition of SHH or purmorphamine (a SHH agonist), then VPA and finally BDNF, GDNF (glial-derived neurotrophic factor), IGF (insulin growth factor), ascorbic acid and cAMP yielded striatal neurons after 47 days [76]. Specifically, of the β-III-tubulin-positive neurons (93% of total cells), 90% expressed GABA, and 90% of these GABAAergic neurons expressed DARPP-32 [76]. DARPP-32 immunopositive cells were observed in vivo, at 4 months after transplantation into an excitotoxic lesioned mouse model, with DARPP-32-positive/GABA-positive neurons making up 58% of total grafted cells. Behavioural analysis revealed improvements in transplanted mice on various tests including the rotarod test of motor control, open-field measures such as crossings and total distance moved, and increased stride length on the Treadscan test for gait analysis. Again, it is important to note that whilst the authors claim no graft overgrowth, the number of total cells in the grafts at the time of analysis was greater than 3 million, whilst only 100,000 cells were transplanted. Taken together, these data showed the successful generation of MSNs that had the capacity to alleviate some of the locomotor deficits seen in this model of HD. Reproduction of this effect has not been reported, despite numerous attempts by others, and so caution must be taken in the interpretation of these findings.

The first report of striatal differentiation from PSCs that utilised the dual SMAD protocol for neural induction, previously mentioned [72], used SB431542, noggin and dorsomorphin, for the initial neural induction phase, with subsequent addition of SHH and DKK-1, and later BDNF [73]. After 45 days in vitro, GABA-positive/DARPP-32-positive cells were observed, and by day 80, 20% of Map2ab+ neurons (51% of total cells) expressed DARPP-32. Following transplantation into a lesioned rat striatum, a modest functional improvement was reported (on an apomorphine-induced rotation test), as early as 3-week post-transplantation, which was maintained at 6 weeks, but fell short of significance at 9-week post-transplantation. Additionally, FoxP1-positive cells and a small number of graft-derived DARPP-32-positive neurons were seen [73]. A total of 4 million cells per graft were reported at 9-week post-transplantation (following transplantation of 500,000 cells), with approximately 2000 DARPP-32-positive cells per graft (0.05%). Again, caution must be conveyed in interpreting these data as the number of animals was low for confirmation of a significant effect, and with respect to the in vivo DARPP-32 staining presented, the numbers seen were very low.

Another study that employed the dual SMAD inhibition protocol using SB431542 and LDN or noggin also looked at the effects of addition of SHH or cycloamine (a SHH antagonist), and Wnt3a, DKK-1 or XAV-939 (the latter two being Wnt pathway inhibitors) [74]. Striatal neuron expressing DARPP-32, calbindin and calretinin were yielded after 45 days in culture, with a combination of XAV-939 (1 μM) or DKK-1 (100 ng/ml), and SHH (50 ng/ml) resulting in optimal numbers of DARPP-32 immunopositive cells (~25% of MAP2-positive neurons). With longer time periods in culture (>60 days), increased expression of DARPP-32 was observed, as well as expression of other striatal neuron markers including CTIP2, dopamine receptors D1 and D2, calbindin and substance P [74]. Analysis of these cells at 5-month post-transplantation showed expression of DARPP-32-positive neurons throughout grafts that co-expressed FoxP1 and CTIP2. In addition, grafts were seen to take over most of the host striatum, although
assessment of proliferative markers, markers of cells from different lineages or total cell numbers were not reported [74].

A novel striatal conversion protocol, still utilising dual SMAD inhibition for the initial neural induction phase, but introducing Activin A in the patterning stage and reporting the redundancy of SHH, resulted in DARPP-32-positive neurons after 36–40 days in vitro [75]. The numbers of DARPP-32-positive neurons yielded in vitro ranged from 20 to 50%, depending on the PSC line being assessed, and expression of striatal markers calbindin and CTIP2 was also reported. Although no functional effect was seen in vivo after transplantation of these cells, the striatal neuronal differentiation reported was considerably better than in previous studies [75]. Most graft-derived cells expressed GABA, and DARPP-32-positive neurons comprised 49% of total graft-derived cells. Other striatal phenotypes observed were calbindin, FoxP2, dopamine receptor D2 and substance P. The proliferative marker, Ki-67, was detected at 4-week post-transplantation, but was absent from grafts at 16-week post-transplantation. Furthermore, the largest graft comprised approximately 500,000 cells (determined by assessment of human nuclei immunostaining), at 16-week post-transplantation, following engraftment of 400,000 cells per graft [75].

The characteristics of human-derived MSNs at an electrophysiological level are not well described. We have previously used calcium imaging analysis to look at neuronal differentiation and functional cellular activity of primary human foetal-derived MSNs [7]. Exposing the in vitro differentiated neurons to various stimuli and neurotransmitter applications resulted in rises in intracellular calcium concentration. Stimuli used included GABA, NMDA, AMPA, kainate, L-glutamate, all of which are indicators of striatal function. A noteworthy finding is that observed following application of GABA, which showed an increase in intracellular calcium and therefore demonstrated a voltage-activated calcium influx in response to a degree of depolarisation. This is indicative of a foetal phenotype, rather than adult. Thus, this study showed that even after 24 days in vitro, the differentiated neurons still exhibited a foetal phenotype [7]. Apart from this preliminary analysis, little is known about the functional capacity of human-derived MSNs in vitro.

In comparison, some of the studies described above that generated MSNs from human PSCs have progressed further in understanding such characteristics. ES cell-derived neurons were reported to be mature and functional after 4 weeks in culture [71]. In this study, where there was no specific patterning towards striatal cell fates, neurons exhibited whole-cell currents including fast, voltage-activated and rapidly inactivating inward currents followed by slowly activated but sustained outward currents, and when stimulated generated action potentials. When differentiation of PSCs was directed towards a striatal MSN phenotype, generation of functional striatal neurons from PSCs has been confirmed [73, 75, 76]. Specifically, generation of GABAergic neurons was confirmed by stimulation with a high-potassium solution and subsequent measurement of the levels of GABA released, which showed that these cells produced a significantly greater amount of GABA than GABA interneurons [76]. In addition, these cells had the potential to generate action potentials following whole-cell patch clamping. Striatal neurons derived using protocols combining the dual SMAD inhibition method for neural induction followed by striatal patterning were shown to have the capacity
to function in a network, forming synapses and showing responsiveness to GABAergic and dopaminergic stimulation [73]. Furthermore, PSC-derived MSNs showed the ability to form GABAergic synapses and exhibited responses to a stimulus and delayed action potential firing typical of striatal MSNs [75]. These are crucial steps in validating the potential of these cells for use in transplantation.

One issue with the use of PSCs as donor cell sources is the exclusivity, initially with respect to neuralisation, and later ‘striatalisation’, of the ‘induction-patterning-differentiation’ protocols applied. Thus, this begs the questions: ‘How heterogeneous is the resultant population with respect to cell types of other, perhaps, unwanted lineages?’ and ‘How much of a problem is this?’ Certainly, the continued presence of undifferentiated PSCs and/or unrestrained proliferative cells in the culture system immediately prior to engraftment makes these cells less attractive as a prospect for transplantation due to the potential risk of uncontrolled overgrowth and even generation of teratomas.

It is clear that the directed differentiation of PSCs in vitro can yield functional striatal MSNs, albeit with varied times in culture and application of different signalling combinations at the different stages of the differentiation process. Transplantation of PSCs into animal models of HD has met with mixed fortunes with the majority of studies exhibiting overgrowth and teratoma formation in the host brain post-transplantation, thus emphasizing the importance of in vivo validation of in vitro cell generation. Identifying a more refined growth factor/signalling molecule cocktail may, perhaps, be a necessary prelude to using these cells for clinical transplantation application. This work is actively ongoing in many laboratories and forms a key part of the European funded ‘Repair HD’ consortium. What is lacking at this point is a detailed understanding of the potential of human foetal-derived MSNs to function, as the analysis to date is limited.

7. Concluding remarks

We have discussed here the current status of neural transplantation in HD and considered the promise shown by clinical trial data, which have provided proof of principle that the approach works in many cases. However, it is evident that there is still a long way to go, and the challenge for generation of successful, efficacious, reproducible transplants is still large. We have highlighted the importance of assessing functional readouts of grafts and not relying solely on histological assessments. Equally, with potential alternative donor cells, it is critical to undertake in vivo assessments of cells differentiated from these sources, understanding that the in vitro data are just a prelude to the necessary in vivo analysis.

Furthermore, we highlighted the limited preclinical data with respect to human-to-rodent investigations, which would advance our understanding of transplanted striatal MSNs derived from both human primary foetal tissue and PSCs. In addition, we see the requirement for future transplant experiments to seek to incorporate neurorehabilitation post-transplantation, in the form of training the graft and also environmental enrichment, as this may well impact on the findings pertaining to the donor cell source.
It appears very probable that an expandable donor cell source will be utilised in future clinical transplant trials, and we have discussed here reports of directed differentiation of such sources to MSNs, albeit with varying degrees of success. However, it is important to continue to gain understanding of human primary foetal striatal cells, including aspects of their development, physiological assessments both in vitro and in vivo, and their ability to generate effective transplants, restoring functional deficits seen in different HD models. This will be the foundation against which all possible alternatives should be compared as part of any preclinical validation.

Acknowledgements

The authors are very grateful to Anne Rosser for guidance and feedback during the preparation of this chapter.

Abbreviations

HD  Huntington's disease
MSN  Medium-sized spiny neurons
WGE  Whole ganglionic eminence
MTOP  Medical termination of pregnancy
STOP  Surgical termination of pregnancy
MRI  Magnetic resonance imaging
UHDRS  Unified HD rating scale
PET  Positron emission tomography
CAPIT-HD  Core Assessment Program for Intracerebral Transplantation in Huntington's Disease
AChE  Acetylcholinesterase
DARPP-32  Dopamine- and cyclic-AMP-regulated phosphoprotein of molecular weight 32 kDa
ES  Embryonic stem
iPS  Induced pluripotent stem
PSC  Pluripotent stem cell
LTP  Long-term potentiation
GABA  Gamma amino butyric acid
SHH  Sonic hedgehog
DKK-1  Dickkopf
BDNF  Brain-derived neurotrophic factor
GDNF  Glial-derived neurotrophic factor
VPA  Valproic acid
dbcAMP  Dibutyryl cyclic AMP
IGF  Insulin growth factor
NMDA  N-methyl-D-aspartic acid
AMPA  α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate
Author details
Sophie V. Precious* and Claire M. Kelly1,2
*Address all correspondence to: precioussv@cf.ac.uk
1 Brain Repair Group, School of Biosciences, Cardiff University, Cardiff, Wales, UK
2 School of Health Sciences, Cardiff Metropolitan University, Cardiff, Wales, UK

References

[1] Dunnett SB, Rosser AE. Cell transplantation for Huntington's disease should we continue? Brain Res Bull. 2007;72:132–147.

[2] Rosser AE, Bachoud-Levi AC. Clinical trials of neural transplantation in Huntington's disease. Prog Brain Res. 2012;200:345–371.

[3] Cisbani G, Cicchetti F. The fate of cell grafts for the treatment of Huntington's disease: the post-mortem evidence. Neuropathol Appl Neurobiol. 2014;40:71–90.

[4] Precious SV, Rosser AE. Producing striatal phenotypes for transplantation in Huntington's disease. ExpBiol Med. 2012;237:343–351.

[5] Dunnett SB, Bjorklund A. Staging and dissection of rat embryos. In: Dunnett SB, Bjorklund A, editors. Neural Transplantation: A Practical Approach. Oxford: IRL Press; 1992. pp. 1–19.

[6] Rosser AE, Dunnett SB. Neural transplantation for the treatment of Huntington's disease. In: Krauss JK, Jankovic J, Grossman RG, editors. Movement Disorders Surgery. Philadelphia: Lippincott, Williams & Wilkins; 2001. pp. 353–373.

[7] Kelly CM, Precious SV, Torres EM, Harrison AW, Williams D, Scherf C, Weyrauch UM, Lane EL, Allen ND, Penketh R, Amso NN, Kemp PJ, Dunnett SB, Rosser AE. Medical terminations of pregnancy: a viable source of tissue for cell re-placement therapy for neurodegenerative disorders. Cell Trans. 2011;20:503–513.

[8] Kopyyov OV, Jacques S, Lieberman A, Duma CM, Eagle KS. Safety of intrastriatalneurotransplantation for Huntington's disease patients. Exp Neurol. 1998;149:97–108.

[9] Bachoud-Lévi A, Bourdet C, Brugières P, Nguyen JP, Grandmougin T, Haddad B, Jény R, Bartolomeo P, Boissé MF, Barba GD, Degos JD, Ergis AM, Lefaucheur JP, Lisovoski F, Pailhous E, Rémy P, Palfi S, Defer GL, Cesaro P, Hantraye P, Peschanski M. Safety and toler-ability assessment of intrastriatal neural allografts in five patients with Huntington's disease. Exp Neurol. 2000;161:194–202.

[10] Rosser AE, Barker RA, Harrower T, Watts C, Farrington M, Ho AK, Burnstein RM, Menon DK, Gillard JH, Pickard J, Dunnett SB, NEST-UK. Unilateral transplantation of human primary fetal tissue in four patients with Huntington's disease: NEST-UK safety report ISRCTN no. 36 485 475. J Neurol Neurosurg Psychiatry. 2002;73:678–685.
[11] Hauser RA, Furtado S, Cimino CR, Delgado H, Eichler S, Schwartz S, Scott D, Nauert GM, Soety E, Sossi V, Holt DA, Sanberg PR, Stoeossl AJ, Freeman TB. Bilateral human fetal striatal transplantation in Huntington's disease. Neurology. 2002;58:687–695.

[12] Reuter I, Tai YF, Pavese N, Chaudhuri KR, Mason S, Polkey CE, Clough C, Brooks DJ, Barker RA, Piccini P. Long-term clinical and positron emission tomography outcome of fetal striatal transplantation in Huntington's disease. J Neurol Neurosurg Psychiatry. 2008;79:948–951.

[13] Capetian P, Knoth R, Maciaczyk J, Pantazis G, Ditter M, Bokla L, Landwehrmeyer GB, Volk B, Nikkhah G. Histological findings on fetal striatal grafts in a Huntington's disease patient early after transplantation. Neuroscience. 2009;160:661–675.

[14] Gallina P, Paganini M, Lombardini L, Mascalchi M, Porfiro B, Gadda D, Marini M, Pinzani P, Salvianti F, Crescioli C, Bucciantini S, Mechi C, Sarchielli E, Romoli AM, Bertini E, Urbani S, Bartolozzi B, De Cristofaro MT, Piacentini S, Saccardi R, Pupi A, Vannelli GB, Di Lorenzo N. Human striatal neuroblasts develop and build a striatal-like structure into the brain of Huntington's disease patients after transplantation. Exp Neurol. 2010;222:30–41.

[15] Bachoud-Lévi AC, Rémy P, Nguyen JP, Brugières P, Lefaucheux JP, Bourdet C, Baudic S, Gaura V, Maison P, Haddad B, Boissé MF, Grandmougin T, Jény R, Bartolomeo P, DallaBarba G, Degos JD, Lisovoski F, Ergis AM, Paihous E, Cesaro P, Hantraye P, Peschanski M. Motor and cognitive improvements in patients with Huntington's disease after neural transplantation. Lancet. 2000;356(9246):1975–1979.

[16] Bachoud-Lévi AC, Gaura V, Brugières P, Lefaucheux JP, Boissé MF, Maison P, Baudic S, Ribeiro MJ, Bourdet C, Remy P, Cesaro P, Hantraye P, Peschanski M. Effect of fetal neural transplants in patients with Huntington's disease 6 years after surgery: a long-term follow-up study. Lancet Neurol. 2006;5(4):303–309.

[17] Barker RA, Mason SL, Harrower TP, Swain RA, Ho AK, Sahakian BJ, Mathur R, Elneil S, Thornton S, Hurrelbrink C, Armstrong RJ, Tyers P, Smith E, Carpenter A, Piccini P, Tai YF, Brooks DJ, Pavese N, Watts C, Pickard JD, Rosser AE, Dunnett SB, the NEST-UK collaboration. The long-term safety and efficacy of bilateral transplantation of human fetal striatal tissue in patients with mild to moderate Huntington's disease. J NeurolNeurosurg Psychiatry. 2013;84:657–665.

[18] Freeman TB, Cicchetti F, Bachoud-Lévi AC, Dunnett SB. Technical factors that influence neural transplant safety in Huntington's disease. Exp Neurol. 2011;227:1–9.

[19] Keene CD, Sonnen JA, Swanson PD, Kopyov O, Leverenz JB, Bird TD, Montine TJ. Neural transplantation in Huntington's disease: long-term grafts in two patients. Neurology. 2007;68:2093–2098.

[20] Keene CD, Chang RC, Leverenz JB, Kopyov O, Perlman S, Hevner RF, Born DE, Bird TD, Montine TJ. A patient with Huntington's disease and long-surviving fetal neural transplants that developed mass lesions. ActaNeuropathol. 2009;117:329–338.
[21] Freeman TB, Cicchetti F, Hauser RA, Deacon TW, Li XJ, Hersch SM, Nauert GM, Sanberg PR, Kordower JH, Saporta S, Isacson O. Transplanted fetal striatum in Huntington's disease: Phenotypic development and lack of pathology. PNAS. 2000;97(25):13877–13882.

[22] Cicchetti F, Saporta S, Hauser RA, Parent M, Saint-Pierre M, Sanberg PR, Li XJ, Parker JR, Chu Y, Mufson EJ, Kordower JH, Freeman TB. Neural transplants in patients with Huntington's disease undergo disease-like neuronal degeneration. PNAS. 2009;106(30):12483–12488.

[23] Cisbani G, Freeman TB, Soulet D, Saint-Pierre M, Gagnon D, Parent M, Hauser RA, Barker RA, Cicchetti F. Striatal allografts in patients with Huntington's disease: impact of diminished astrocytes and vascularization on graft viability. Brain. 2013;136:433–443.

[24] Deacon TW, Pakzaban P, Isacson O. The lateral ganglionic eminence is the origin of cells committed to striatal phenotypes: neural transplantation and developmental evidence. Brain Res. 1994;668:211–219.

[25] Olsson M, Bjorklund A, Campbell K. Early specification of striatal projection neurons and interneuronal subtypes in the lateral and medial ganglionic eminence. Neuroscience. 1998;84:867–876.

[26] Grasbon-Frodl EM, Nakao N, Lindvall O, Brundin P. Phenotypic development of the human embryonic striatal primordium: a study of cultured and grafted neurons from the lateral and medial ganglionic eminences. Neuroscience. 1996;73(1):171–183.

[27] Nakao N, Grasbon-Frodl EM, Widner H, Brundin P. DARPP-32-rich zones in grafts of lateral ganglionic eminence govern the extent of functional recovery in skilled paw reaching in an animal model of Huntington's disease. Neuroscience 1996;74: 959–970.

[28] Olsson M, Campbell K, Victorin K, Bjorklund A. Projection neurons in fetal striatal transplants are predominantly derived from the lateral ganglionic eminence. Neuroscience. 1995;69:1169–1182.

[29] Watts C, Brasted PJ, Eagle DM, Dunnett SB. Embryonic donor age and dissection influences striatal graft development and functional integration in a rodent model of Huntington's disease. Exp Neurol. 2000;163:85–97.

[30] Fricker RA, Torres EM, Dunnett SB. The effects of donor stage on the survival and function of embryonic striatal grafts in the adult rat brain. I. Morphological characteristics. Neuroscience. 1997;79:695–710.

[31] Evans AE, Kelly CM, Precious SV, Rosser AE. Molecular regulation of striatal development: a review. Anat Res Int. 2012;Article ID:106529. doi:10.1155/2012/106529

[32] Naimi S, Jeny R, Hantraye P, Peschanski M, Riche D. Ontogeny of human striatal DARPP-32 neurons in fetuses and following xenografting to the adult rat brain. Exp Neurol. 1996;137:15–25.

[33] Onorati M, Castiglioni V, Biasci D, Cesana E, Menon R, Vuono R, Talpo F, Laguna Goya R, Lyons PA, Bullamante GP, Muzio L, Martino G, Toselli M, Farina C, Barker RA, Biella
G, Cattaneo E. Molecular and functional definition of the developing human striatum. Nat Neurosci. 2014;17(12):1804–1815.

[34] Watts C, Dunnett SB, Rosser AE. Effect of embryonic donor age and dissection on the DARPP-32 content of cell suspensions used for intrastriatal transplantation. Exp Neurol. 1997;148:271–280.

[35] Pundt LL, Kondoh T, Conrad JA, Low WC. Transplantation of human striatal tissue into a rodent model of Huntington’s disease: phenotypic expression of transplanted neurons and host-to-graft innervation. Brain Res Bull. 1996;39(1):23–32.

[36] Pundt LL, Kondoh T, Conrad JA, Low WC. Transplantation of human fetal striatum into a rodent model of Huntington’s disease ameliorates locomotor deficits. Neurosci Res. 1996;24:415–420.

[37] Sanberg PR, Borlongan CV, Koutouzis TK, Norgren RB Jr, Cahill DW, Freeman TB. Human fetal striatal transplantation in an excitotoxiclesioned model of Huntington’s disease. Ann N Y Acad Sci. 1997;831:452–60.

[38] Lelos MJ, Roberton VH, Vinh NN, Harrison C, Peter Eriksen P, Torres EM, Clinch SP, Rosser AE, Dunnett SB. Direct comparison of rat- and human-derived ganglionic emi
- nence tissue grafts on motor function. Cell Transplant. 2016;25:665–675.

[39] Watts C, Brasted PJ, Dunnett SB. The morphology, integration, and functional efficacy of striatal grafts differ between cell suspensions and tissue pieces. Cell Transplant. 2000;9(3):395–407.

[40] Brasted PJ, Watts C, Robbins TW, Dunnett SB. Associative plasticity in striatal transplants. PNAS. 1999;96:10524–10529.

[41] Döbrössy MD, Dunnett SB. Training specificity, graft development and graft-mediated functional recovery in a rodent model of Huntington’s disease. Neuroscience. 2005;132:543–552.

[42] Döbrössy MD, Dunnett SB. Optimising plasticity: environmental and training associated factors in transplant-mediated brain repair. Rev Neurosci. 2005;16(1):1–21.

[43] Döbrössy MD, Dunnett SB. Environmental housing and duration of exposure affect striatal graft morphology in a rodent model of Huntington’s disease. Cell Transplant. 2008;17:1125–1134.

[44] Döbrössy MD, Nikkah G. Role of experience, training, and plasticityin the functional efficacy of striatal transplants. Prog in Brain Res. 2012;200:303–28.

[45] Döbrössy MD, Dunnett SB. Environmental enrichment affects striatal graft morphology and functional recovery. Eur J Neurosci. 2004;19:159–168.

[46] Döbrössy MD, Dunnett SB. Morphological and cellular changes within embryonic striatal grafts associated with enriched environment and involuntary exercise. Eur J Neurosci. 2006;24:3223–3233.
[47] Mazzocchi-Jones, D, Döbrössy MD, Dunnett SB. Embryonic striatal grafts restore bi-directional synaptic plasticity in a rodent model of Huntington’s disease. Eur J Neurosci. 2009;30:2134–2142.

[48] Mazzocchi-Jones, D, Döbrössy MD, Dunnett SB. Environmental enrichment facilitates long-term potentiation in embryonic striatal grafts. Neurorehabil. Neural Repair. 2011;26(6):548–557.

[49] Kelly CM, Precious SV, Scherf C, Penketh R, Amso NN, Battersby A, Allen ND, Dunnett SB, Rosser AE. Neonatal desensitization allows long-term survival of neural xenotransplants without immunosuppression. Nat Methods. 2009;6(4):271–273.

[50] Hurelbrink CB, Armstrong RJ, Barker RA, Dunnett SB, Rosser AE. Hibernated human fetal striatal tissue: successful transplantation in a rat model of Huntington’s disease. Cell Transplant. 2000;9(6):743–749.

[51] Hurelbrink CB, Tyers P, Armstrong RJ, Dunnett SB, Barker RA, Rosser AE. Long-term hibernation of human fetal striatal tissue does not adversely affect its differentiation in vitro or graft survival: implications for clinical trials in Huntington’s disease. Cell Transplant. 2003;12(7):687–95.

[52] Kelly CM, Dunnett SB, Rosser AE. Medium spiny neurons for transplantation in Huntington’s disease. Biochem Soc Trans. 2009;37:323–328.

[53] Zietlow R, Pekarik V, Armstrong RJ, Tyers P, Dunnett SB, Rosser AE. The survival of neural precursor cell grafts is influenced by in vitro expansion. J Anat. 2005;207(3):227–240.

[54] Anderson L, Burnstein RM, He X, Luce R, Furlong R, Foltynie T, Sykacek P, Menon DK, Caldwell MA. Gene expression changes in long term expanded human neural progenitor cells passaged by chopping lead to loss of neurogenic potential in vivo. Exp Neurol. 2007;204:512–524.

[55] Jain M, Armstrong RJE, Tyers P, Barker RA, Rosser AE. GABAergic immunoreactivity is predominant in neurons derived from expanded human neural precursor cells in vitro. Exp Neurol. 2003;182:113–123.

[56] Caldwell M, He X, Wilkie N, Pollack S, Marshall G, Wafford KA, Svendsen CN. Growth factors regulate the survival and fate of cells derived from human neurospheres. Nat Biotechnol. 2001;19(5):475–479.

[57] Zietlow R, Precious SV, Kelly CM, Dunnett SB, Rosser AE. Long-term expansion of human foetal neural progenitors leads to reduced graft viability in the neonatal rat brain. Exp Neurol. 2012;235(2):563–573.

[58] Kelly CM, Precious SV, Penketh R, Amso N, Dunnett SB, Rosser AE. Striatal graft projections are influenced by donor cell type and not the immunogenic background. Brain. 2007;130:1317–1329.
[59] Precious SV, Zietlow R, Dunnett SB, Kelly CM, Rosser AE. Human fetal-derived stem cells for cell replacement therapy in Huntington's disease. 2016 (Submitted).

[60] Martin GR. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proc Natl Acad Sci U S A. 1981;78:7634–7638.

[61] Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. Nature. 1981;292:154–156.

[62] Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. Science. 1998;282:1145–1147.

[63] Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006;126:663–676.

[64] Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007;131:861–872.

[65] Arlotta P, Molyneaux BJ, Jabadon D, Yoshida Y, Macklis JD. Ctip2 controls the differentiation of medium spiny neurons and the establishment of the cellular architecture of the striatum. J. Neurosci. 2008;28:622–632.

[66] Precious SV, Kelly CM, Reddington AE, Vinh NN, Stickland RC, Pekarik V, Scherf C, Jeyasinghan R, Glasbey J, Holeiter M, Jones L, Taylor MV, Rosser AE. FoxP1 marks medium spiny neurons from precursors to maturity and is required for their differentiation. Exp Neurol. 2016;282:9–18.

[67] Perrier AL, Tabar V, Barberi T, Rubio ME, Bruses J, Topf N, Harrison NL, Studer L. Derivation of midbrain dopamine neurons from human embryonic stem cells. PNAS. 2004;101:12543–12548.

[68] Aubry L, Bugi A, Lefort N, Rousseau F, Peschanski M, Perrier AL. Striatal progenitors derived from human ES cells mature into DARPP32 neurons in vitro and in quinolinic acid-lesioned rats. PNAS. 2008;105:16707–16712.

[69] Joannides A, Fiore-Heriche C, Westmore K, Caldwell M, Compston A, Allen N, Chandran S. Automated mechanical passaging: a novel and efficient method for human embryonic stem cell expansion. Stem Cells. 2006;24:230–235.

[70] Joannides AJ, Fiore-Heriche C, Battersby AA, Athauda-Arachchi P, Bouhon IA, Williams L, Westmore K, Kemp PJ, Compston A, Allen ND, Chandran S. A scaleable and defined system for generating neural stem cells from human embryonic stem cells. Stem Cells. 2007;25:731–737.

[71] Joannides AJ, Webber DJ, Raineteau O, Kelly C, Irvine KA, Watts C, Rosser AE, Kemp PJ, Blakemore WF, Compston A, Caldwell MA, Allen ND, Chandran S. Environmental sig-
nals regulate lineage choice and temporal maturation of neural stem cells from human embryonic stem cells. Brain. 2007;130:1263–1275.

[72] Chambers SM, Fasano CA, Papapetrou EP, Tomishima M, Sadelain M, Studer L. Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. Nat Biotechnol. 2009;27:275–280.

[73] Delli-Carri A, Onorati M, Lelos MJ, Castiglioni V, Faedo A, Menon R, Camnasio S, Vuono R, Spaiaardi P, Talpo F, Toselli M, Martino G, Barker RA, Dunnett SB, Biella G, Cattaneo E. Developmentally coordinated extrinsic signals drive human pluripotent stem cell differentiation toward authentic DARPP-32+ medium-sized spiny neurons. Development. 2013;140:301–312.

[74] Nicoleau C, Varela C, Bonnefond C, Maury Y, Bugi A, Aubry L, Viegas P, Bourgois-Rocha F, Peschanski M, Perrier AL. Embryonic stem cells neural differentiation qualifies the role of Wnt/b-catenin signals in human telencephalic specification and regionalization. 2013;31:1763–1774.

[75] Arber C, Precious SV, Cambray S, Risner-Janiczek JR, Kelly C, Noakes Z, Fjodorova M, Heuer A, Ungless MA, Rodriguez TA, Rosser AE, Dunnett SB, Li M. Activin A directs striatal projection neuron differentiation of human pluripotent stem cells. Development. 2015;142:1375–1386.

[76] Ma L, Hu B, Liu Y, Vermilyea SC, Liu H, Gao L, Sun Y, Zhang X, Zhang SC. Human embryonic stem cell-derived GABA neurons correct locomotion deficits in quinolinic acid-lesioned mice. Cell Stem Cell. 2012;10:455–464.