

Position Paper

Stem Cell-Derived Dopamine Neurons: Will They Replace DBS as the Leading Neurosurgical Treatment for Parkinson's Disease?

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Pre-press 26 July 2021

Abstract. The use of stem cell-derived dopamine neurons or deep brain stimulation (DBS) represents two alternative approaches to treat Parkinson's Disease. DBS is a widely used FDA-approved treatment and stem cell-derived dopamine neuron replacement has now evolved to the first in-human clinical trials. In this debate, we discuss which of these approaches will evolve to be the treatment of choice for Parkinsonian patients in the future.

Keywords: Stem cells, dopamine neurons, DBS, brain repair

STEM CELL-DERIVED DOPAMINE NEURONS WILL REPLACE DBS AS THE LEADING NEUROSURGICAL TREATMENT FOR PARKINSON'S DISEASE: ROGER A. BARKER AND ANDERS BJÖRKLUND

Parkinson's disease (PD) is a complex heterogeneous disorder for which the aetiology, in the majority of cases, is unknown [1]. The pathology centers on

the accumulation of alpha synuclein in populations of nerve cells both within the central nervous system as well as outside in the autonomic and enteric nervous systems. However critical to the expression of many features of PD is the loss of the dopaminergic innervation from the A9 nigral neurons to the striatum, in particular the putamen. How do we know this? In the first instance, this defining feature of the disease has been shown at post-mortem, as well as by PET and SPECT imaging, and remains the gold standard for diagnosing PD [2]. However, this only tells us that the nigral dopamine cells and their projections are lost, it does not tell us that this leads to the expression of many of the features of PD. This though has been proven through the effectiveness of dopaminergic drugs in ameliorating many of the early features of PD [3]. Indeed, when patients with early-stage disease receive such agents they return almost

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to normal and the therapeutic benefit can in many cases be maintained for many years.

So why is this important for our debate? The efficacy of dopaminergic drug treatment provides a compelling rationale for the use of dopamine cell therapies to restore striatal dopamine neurotransmission. This approach should, at the very least, work as well as oral dopamine therapies with the advantage that the cells can be targeted to the site where dopamine is most needed, namely the striatum. This has the benefit that it will prevent the patient from experiencing many of the neuropsychiatric and autonomic off target effects that can occur with oral dopaminergic therapies, sometimes disastrously (e.g. [4]). In addition, if dopamine cell replacement uses neurons that release dopamine synaptically in a physiological fashion then it would also get around the problems seen with chronic L-dopa use and the generation of L-dopa-induced dyskinesias (LIDs) [5, 6]. Oral L-dopa treatment delivers dopamine in a pulsatile, non-physiological fashion, and the duration and dose of its administration determine when LIDs develop [7]; the development of this side effect is indeed one of the major indications for patients to undergo DBS surgery [8]. If stem cell-derived dopamine neuron transplants work well they will have the potential to make deep brain stimulation (DBS) therapy redundant as the grafted patients will no longer develop significantly disabling LIDs, thus replacing DBS as the leading neurosurgical therapy for PD!

ADVANTAGES FACILITATING THE SWITCH FROM DBS SURGERY TO CELL IMPLANTATION

Dopamine neuron transplants are attractive alternatives to DBS for a number of reasons:

1. They are working in a way that makes more intuitive sense, replacing structurally and functionally a key population of neurons lost to the disease – DBS is essentially only symptom-correcting, leaving the underlying damage untouched;
2. They restore damaged circuitry and bring basal ganglia network activity back to a more normal physiological state, and obviate the need for any dopaminergic drugs and the complications that come with them (see above) - DBS is just trying to correct an aberrant circuit abnormally, can only ever reduce drug intake, and never prevents complications from their continued use;
3. It is a one-off procedure with a short period of immunosuppression with a marginal transient risk of infection – DBS requires battery changes with a clear physical and somewhat unsightly reminder of an intervention, as is evident by the wires and batteries that are permanently to be seen subcutaneously in patients. This brings with it continued infective risks for the patients with DBS which is not so for patients with transplants;
4. They should over time become cheaper to make and easier to deliver, which will facilitate the switch from DBS surgery to cell implantation. The 16-day differentiation protocol that has been adopted by most teams means large number of cells can be made cheaply and efficiently, stored and delivered from a cryopreserved state;
5. While the surgical implantation of the cells carries an operative risk, this is likely to be less than for DBS because the cells will be delivered with relatively simple devices to a large structure (the putamen) which is different to DBS where small structures, deep within the brain (e.g. subthalamic nucleus), are targeted with indwelling electrodes;
6. The next generation of cells will be immunologically silent, e.g. by knocking out major and minor MHC antigens, and they may also be engineered to prevent them from ever being affected by the disease- e.g. through making alpha synuclein knock out cells.

HOW QUICKLY WILL THIS HAPPEN?

Even if the advantages of dopamine cell replacement, in theory, should remove the need for DBS, we first need to ask whether it is as effective, and thus clinically truly competitive to DBS. To begin with, it is important to stress that neither treatment cures PD nor is it suitable for everyone with this condition. However, it is very likely that the patients currently selected as the optimal candidates for DBS will also be the optimal ones for dopamine cell therapy - namely younger patients with an excellent response to dopaminergic therapies and with little or no major cognitive or non-motor/non-dopaminergic pathology as expressed clinically. Thus, the question we have to ask ourselves to prove our case is, whether there is any evidence that dopamine cell therapies can work very well in patients with PD? The answer to this is a clear yes.

We have previously reviewed this literature [9] but for our discussion here, the key evidential points hinge on whether dopamine neurons can survive and function long-term in the PD brain and, if so, produce a benefit that is equivalent or better than that seen with DBS. Whilst we accept that the data from trials using human fetal dopamine cells have produced inconsistent results there is no doubt that in the best cases the grafts have:

- restored patients back to pre-diagnosis levels of motor control in the absence of dopaminergic drugs, and that this improvement has been maintained over more than a decade [10];
- restored dopaminergic innervation of the striatum back to normal, as seen on PET imaging and at post-mortem [11].

The fact that this has not been obtained in all cases relates much to the quality, preparation and handling of the fetal tissue, given that these trials have used a non-standardised approach with each patient receiving transplants made from their own unique collection of fetal ventral midbrain tissue. Thus, the dose and quality of cells given to patients in these trials have varied enormously given the wide difference in the number and age of fetuses that have been used to make the implantable cell suspensions. This, coupled to the different ways by which these cells were actually engrafted into the brain and the differing levels of protection thereafter (most notably the type and extent of host immunosuppression employed post grafting), means that it is hardly surprising that the results are so variable. Of course, this variability would be a problem going forward, especially compared to the standardization of therapy that comes with modern DBS, if the approach had to rely on the use of fetal tissue. However, this will not be the case, as the new generation of transplants will be derived from stem cell sources and the generation of well-defined homogeneous batches of cells. As such, all patients will now be able to receive the same dose of the same well-characterized cell product. Coupled to the use of a standardised neurosurgical approach with a new generation of specially designed implantation devices this should ensure that all patients receive essentially the same treatment. Whilst the exact optimal instrument for engrafting cells to the brain is yet to be defined, there is no doubt that this will be achieved (as was the case for DBS in the 1990s and early part of this century) given progress in related fields, such as neuro-oncology and gene therapy trials [12–14].

In summary, given the biological rationale of this one-time restorative intervention, the cheap and efficient production of the cells, their ease of delivery, and the long-lasting benefits they will offer, we are convinced that stem cell-derived dopamine neurons will inevitably replace DBS as the leading neurosurgical treatment for PD. Of course, this is all predicated on the grounds that the ongoing and future clinical trials using stem cell-derived dopamine neuron transplants show an efficacy that is consistent and as good as that obtained with the best fetal transplants. This has yet to be proven but there is no reason to think that authentic midbrain dopamine cells made from human stem cell sources should not fare as well as the best “real” fetal midbrain dopamine cells transplanted in trials to date. Obviously, the clinical stem cell-based trials which now are being initiated at several centers throughout the world should not be rushed or prejudged and should be done with proper blinding and equipoise on the part of those conducting this work. This is especially important as previous work in the field of restorative therapeutics has often sought to take short cuts with detrimental effects to all, including the patients and their families. Something that DBS surgery has not been immune to either!

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STEM CELL-DERIVED DOPAMINE NEURONS WILL NOT REPLACE DBS AS THE LEADING NEUROSURGICAL TREATMENT FOR PARKINSON'S DISEASE: STEVEN J. FRUCHT AND CLIVE N. SVENDSEN

Deep brain stimulation (DBS) has emerged as the leading neurosurgical treatment for Parkinson's disease (PD). Since its inception in 1989 [1], the utility of this surgical approach in carefully selected patients has been demonstrated in numerous clinical trials [2]. DBS was quickly approved by the FDA in the USA as a validated treatment and adopted in PD clinics throughout the world. DBS reliably dampens or even eliminates medication-refractory tremor in PD. In patients who suffer from motor fluctuations (needing to take multiple doses of levodopa per day) or troublesome dyskinesias (involuntary movements fueled by levodopa), DBS of the subthalamic nucleus (STN) or globus pallidus internus reliably allows the total levodopa dose to be substantially reduced, eliminating dyskinesias and providing a smooth, continuous benefit. For this reason, movement disorder neurologists have come to view DBS as "electronic levodopa". Clinical features that improve with levodopa reliably improve with DBS, while medication refractory features (freezing, pos-

tural instability) remain unaffected. With continual optimization in surgical technique, directional stimulation with improved lead design, and the developing ability to perform closed loop stimulation (for real-time internal feedback based on patient activity and need), the safety and efficacy of this procedure continue to evolve and increase, offering an important treatment option for advanced PD patients [2].

In fact, neurologists who have joined the field of movement disorders in the last two decades may not recognize that DBS has already irreversibly altered the landscape of treatment for advanced PD patients. Additionally, as highlighted in a recent review [3], rat and nonhuman primate PD models suggest that STN-DBS may also protect against neuronal loss and reduce motor dysfunction. This is in contrast to most other treatment options that do not provide a neuroprotective effect. While clinical studies provide only limited support for a similar disease-modifying neuroprotective effect in patients [4, 5], this may be because STN-DBS surgery is typically performed several years following diagnosis, long after dopaminergic terminals are lost. Future STN-DBS studies performed earlier in disease may lead to more dramatic neuroprotective effects, although this remains to be established.

However, despite many achievements, DBS is not a good candidate for all patients, for example those with cognitive impairment, significant and untreated psychiatric comorbidities such as hallucinations or psychosis, and patients unable to keep appointments for follow-up monitoring [6]. In addition, DBS does not address some of the most troublesome symptoms in advanced PD such as freezing of gait, apathy, and cognitive decline. Thus patients who will benefit the most from DBS need to be carefully selected and surgical centers that obtain the best results focus on these candidates. Finally, DBS comes with small but significant upfront and long-term risks. These include a very small risk of bleeding with electrode implantation, usually without sequelae and rarely with disastrous consequences, as well as lead infection, lead fracture and pulse generator malfunction. However, on balance the availability and benefits of DBS far outweigh the negatives. These merits are so significant they have actually limited the development of new interventions, as proposed therapeutics are often compared to DBS, and approval is based on risks and benefits in comparison to this proven, safe and effective alternative. Therefore, the bar is set very high for fetal-derived dopamine neurons to

replace DBS as the leading neurosurgical treatment for PD.

The idea of replacing dopamine neurons lost in PD through fetal sources originated in the early 1980s, pioneered by the Bjorklund and Lindvall groups in Sweden [7, 8]. In the early days, developing mesencephalon from aborted human fetal tissue was used as a source of dopamine neurons, transplanted into the striatum of patients with PD. A number of clinical trials in both Sweden and the USA showed that fetal dopamine neurons could survive, integrate into the striatum, and release dopamine [9]. More recently, embryonic and induced pluripotent stem cells (iPSCs) have been used as a source of dopamine neurons, and in one recent trial autologous cells survived without immune suppression in the striatum based on imaging of dopamine release [10]. However, in contrast to DBS where the benefits of the procedure were obvious to clinician and patient, dopamine neuron replacement via fetal transplants has had a far more uncertain course. While early small patient studies were exciting for the field, showing for the first time that fetal-derived dopamine neurons could survive in the brain [11, 12], trials powered to demonstrate clinical benefits were surprisingly disappointing [13]. Trials produced variable outcomes based on conventional UPDRS score changes – some positive [14, 15] and others negative [16–18]. Questions arose about how to prepare the fetal tissue prior to transplantation, the optimal graft location and duration of immunosuppression. A concerning finding in these trials was the unexpected and serious adverse event of graft-induced or “runaway” dyskinesias, as reviewed very recently [19]. Trials worldwide have shown a range from 15% to over 50% of patients experienced these severe uncontrollable movements as a side-effect of the transplant [16, 18, 20, 21]. Interestingly, in some cases these patients required treatment with DBS when all other methods of controlling involuntary movements failed [19, 22] – in further support of our position in this debate! It is certainly disconcerting that fetal transplants cannot be removed in the event of dyskinesias or other risks, in contrast to DBS that has safety measures including adjustment of electrode position and stimulator settings, stimulator shut-off or complete removal in the rare case of infection. Thus the tenant of “do no harm” and the Hippocratic oath can be more easily applied with DBS but not fetal cell transplants. For all of these reasons, in contrast to DBS, the FDA has not yet approved any dopamine cellular product for use in patients.

This has forced the transplant field to move back a few steps and work out what happened in these early clinical trials. For example, what may have caused such horrific dyskinetic side-effects? Dyskinesias were not reported in either rodent or nonhuman primate preclinical studies using identical fetal tissue preparations, making them very hard to model in animals. Ultimately studies from Sweden did develop a rodent model, which demonstrated that contamination of the dopamine transplants with serotonergic neurons might be the main problem [23]. This was subsequently confirmed in patients and selective serotonin receptor agonists attenuated the graft-induced dyskinesias [24], but unfortunately they do not always work. A more significant and perplexing reason for the dyskinesias may be the location of the transplant. During human development, dopamine neurons project fibers from the substantia nigra to the striatum, when distances are short and developmental cues guide outgrowth and innervation. This is in contrast to most trials, in which dopamine grafts have been placed ectopically in the striatum, rather than in the nigra where the dopamine neurons are lost.

Attempts to transplant the dopamine neurons into the substantia nigra showed that the cells could survive and in rare cases lead to anatomical and functional recovery in a rodent model of PD [25]. However, extension of new axons to reform the lesioned nigrostriatal pathway appears to be limited to neonatal rodent [26], and to date in primates has required bridging procedures with double grafting or growth factors [27–29]. While a trial has delivered fetal midbrain cells to the patient substantia nigra, this has been in only one case and axonal outgrowth to the striatum was not assessed [30]. Ectopic transplantation of dopamine neurons assumes that there is reconstruction of the circuitry within the striatum [31], though, it is unlikely that a dopamine graft can recapitulate the complex nigrostriatal circuit for accurate modulation of dopamine release to provide correct transmission of corticothalamic signaling and movement. Indeed, positron emission tomography studies indicate that unbalanced dopaminergic function may be involved in dyskinesias after neuronal transplantation [32]. Grafting dopamine neurons is further complicated given recent post-mortem studies showing that some of new neurons themselves may be susceptible to the ongoing pathological changes [33]. Finally, fetal transplants remain uncertain in regard to ethics, practicality and availability. For example, some countries still do not permit fetal-derived transplants and a recent multicenter

observational study in Europe with a TRANSEURO trial [34] stalled, possibly due to lack of access to fetal tissue. We of course recognize that new technologies using embryonic stem cells or autologous iPSCs [10, 35] may be more practical and perhaps overcome some of the problems of fetal tissue-derived dopamine neurons. However, significant biomanufacturing and cell stability challenges remain for the field, and the ability of stem cell-derived dopamine neurons to fully mature and establish normal host connections in the human brain is not yet proven.

Even if all of these significant challenges are overcome, modern understanding of the cellular mechanisms underlying PD casts doubt that simply placing exogenous grafts into one location in the brain will alter the course of the illness. We now know that rather than thinking of PD as a highly selective degenerative disorder affecting just the nigrostriatal pathway, PD affects multiple cell types and regions within the brain. In fact, the majority of dopamine neurons degenerate long before patients are ever functionally impaired or diagnosed in clinic, with some studies showing that the majority of dopaminergic terminals are lost within 4 years of PD diagnosis [36]. Other regions of the brain known to be affected in PD include the STN, with overactive glutamatergic projections originating in the STN possibly contributing to dopamine neuron excitotoxicity [37]. As well, there is early degeneration of the locus coeruleus leading to noradrenergic dysfunction [38]. None of these are likely to be affected by a dopamine neuron transplant.

In conclusion we posit that dopamine neuron transplants is very unlikely to replace DBS as the leading neurosurgical treatment for PD. DBS is an established, safe and highly effective surgical technique that has already improved the lives of hundreds of thousands of PD patients throughout the world. Dopamine neuron transplants as a therapeutic approach remains a fascinating area of clinical research led by outstanding groups of scientists and clinicians – but in our view and for the reasons outlined above, is unlikely to replace DBS in the foreseeable future.

ACKNOWLEDGMENTS

We thank Dr. Soshana Svendsen for critical input and editing of this commentary.

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REBUTTAL FROM ROGER A. BARKER AND ANDERS BJÖRKLUND

We read with interest the article by Frucht and Svendsen supporting their view that DBS surgery will remain the leading neurosurgical treatment of PD, rather than transplants of stem cell-derived dopamine (DA) neurons. They make a number of valid criticisms about the use of transplants of human fetal ventral midbrain (VM) tissue as a treatment for patients with PD, but of course this is not really relevant to the question being asked here - the use of stem cell-derived DA neurons. It was for this reason that we discussed DBS and not lesion surgery for PD - the precursor of DBS! Fetal VM grafts have shown proof-of-principle around cell survival, efficacy and circuit reconstruction in the PD brain, in much the same way that lesion surgery showed the critical role of globus pallidus and subthalamic nucleus (STN) in the expression of some motor features of PD. Lesion surgery had many problems associated with it, not least long-term therapeutic failure, but this did not stop its successor, DBS, from becoming a popular and effective therapy for PD - albeit one with a limited future, in our opinion. Namely, DBS will continue in the near future to be an important complement to drug

therapy, but the use of this complex stimulator technology will inevitably be replaced by something else, equally or more effective and much more convenient for the patient.

In this perspective, cell replacement therapy holds great promise. While DBS is limited to the correction of the symptoms caused by DA neuron loss, DA neuron transplants correct the deficit, *i.e.*, replace the lost DA neurons, reinstate DA neurotransmission and restore basal ganglia circuitry function. Indeed, DA transplants have been shown to restore movement-related cortical activation [1] and there is also experimental evidence that they can normalise STN firing and beta oscillatory activity much in the same way as DBS does [2, 3].

Many of Frucht and Svendsen's arguments against cell therapy are specific for human fetal VM grafts, such as the contaminating 5HT neurons and the dyskinetic side-effects. We argue that this is not relevant for the stem cell-derived DA cell products that will be available as a standardised, defined populations of cells with none of the variability and contaminants that characterise the fetal VM transplants. In addition, the concern around the spread of pathology to the transplants does not represent much of a problem - it occurs after more than a decade, is limited to a fraction of the grafted DA neurons, and does not appear to affect the functionality of the graft. If needed, this could also be avoided through the use of cells engineered to have a knock out of endogenous alpha-synuclein.

It was also good to read that the authors accept that there are limitations as to what DBS can achieve clinically, and that this therapy is not without side-effects. Indeed, there are problems with DBS, not only perioperatively and the ongoing risks of infection and device failure, but also in the worsening of apathy symptoms [4] and the long-term cognitive, and in some cases speech, deficits linked to white matter damage along the implanted electrode track [5, 6]. With DA cell therapies these risks are simply not there, as the transplant is a one-off procedure with no long-term infective or cognitive risks. In addition, the patients will not be left with a constant reminder of their condition as is the case with the presence and maintenance of the wires and batteries driving the DBS.

Finally, Frucht and Svendsen argue that treating only the dopaminergic cell loss with transplants will not cure patients with PD given its disseminated pathology. We agree, but this was not what we were debating and the same argument holds for placing an

electrode in one nucleus of the brain and stimulating it. At least with cells we are rebuilding part of the lost circuits of PD and with time we may be able to combine cell therapy with a neuroprotective or disease modifying intervention – the grafted cells replacing the lost DA neurons and a therapeutic delivered to block further disease progression!

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REBUTTAL FROM STEVEN J. FRUCHT AND CLIVE N. SVENDSEN

We read with interest the elegant argument put forth by Barker and Bjorklund that stem cell-derived dopamine neurons will replace DBS as the leading neurosurgical treatment for PD. We will address their arguments in turn. The central tenet of their argument relies on the "best case" patients who have received fetal tissue transplants and show good improvements in their Parkinson's disease – and that with better dopamine neurons from stem cells these cases can become the norm for the field. However, these successful cases are in fact rare. Furthermore, several similar double blind clinical trials with good graft survival have shown no effects which suggests an

alternative possibility – that the well controlled clinical trials did not meet their endpoint and dopamine neuron transplants simply do not work. The few cases that have shown graft survival and clinical effects may be an anomaly and represent placebo effects, outlier patients or atypical Parkinson's disease. While targeting dopamine delivery to the striatum using fetal or stem cell-derived dopamine neurons seems like a good idea (though ectopic location remains a shortcoming), we feel it goes a little too far when stated that this would “get around the problems seen with chronic levodopa use and the generation of dyskinesias”. In fact, the exact opposite occurred in the most carefully performed blinded trials, with runaway dyskinesias as an unanticipated and serious adverse event. This unexpected and as-yet incompletely understood phenomenon is enough in our view to require significantly more studies before performing larger trials (similar to those already completed for DBS which led to FDA approval).

The authors list six reasons that cell implantation will replace DBS—we address them in turn:

1. We believe it unlikely that artificially implanted dopamine neurons can recreate the complex connectivity of the denervated PD striatum. Whereas DBS actually works by resetting the circuitry and restoring normal outflow from the STN.
2. The complications of dopaminergic drugs that the authors reference, are much less of an issue in clinical practice in the 21st century. Movement disorder neurologists are now much better skilled at balancing administration of levodopa, agonists and ancillary agents.
3. Advances in DBS surgery, including smaller pulse generators, rechargeable batteries and sophisticated plastic surgical approaches allow most DBS patients to go about their lives with no one aware of their past surgery. The infection risk from DBS electrodes is actually quite small, and usually manageable.
4. Given the behavior of pharmaceutical companies pricing one-off interventions such as gene therapy as high as \$2.1M for spinal muscular atrophy, we are less optimistic that the price point for cell transplantation will be cheaper than DBS.
5. The surgical risks of DBS of the STN are low, and perhaps much lower than transplantation of a non-autologous cell material sourced from fetal tissue or in some cases from pluripotent cells.
6. The ability to definitively avoid immune reaction to implanted cells and the prevention of Lewy body formation in implanted cells remains unresolved.

For those who still think that cell therapy will outperform DBS in the near future, we encourage them to spend a day in clinic following a skilled neurologist who cares for DBS patients. We believe that the improvements in quality of life and the obvious satisfaction of patients and their families with the procedure will be self-evident.