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REVIEW

Functions of the nigrostriatal dopaminergic synapse and the use of neurotransplantation in Parkinson's disease

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Abstract While pharmaceutical options remain the overwhelmingly accepted treatment of choice for neurological and psychiatric diseases, significant accomplishments in regenerative neuroscience research have demonstrated the potential of cellular and synaptic functional repair in future therapies. Parkinson's disease stands out as an example in which repair by dopaminergic neurons appears a viable potential therapy. This article describes the basic neurobiological underpinnings of the rationale for cell therapy for Parkinson's disease and the challenges ahead for the use of regenerative medicine in the treatment for this disease.

Keywords Parkinson's · Movement disorders · Neurotransplantation · Foetal grafting · Dyskinesias · Normal striatum

Introduction

While pharmaceutical options remain the overwhelmingly accepted treatment of choice for neurological and psychiatric diseases, significant accomplishments in regenerative neuroscience research have demonstrated the potential of cellular and synaptic functional repair in future therapies. Parkinson's disease (PD) stands out as an example in which repair by dopaminergic neurones appears a viable potential therapy. This article describes the basic neurobiological underpinnings of the rationale for cell therapy for Parkinson's disease and the challenges ahead for the use of regenerative medicine in the treatment for this disease.

Nigrostriatal dopaminergic projections are essential regulators of motor circuits. In Parkinson's disease, a specific population of midbrain neurones projecting from substantia nigra pars compacta (SNpc) to the dorsolateral striatum progressively degenerate. Loss of these dopaminergic projections, classified as A9 neurones, results in bradykinesia and rigidity classically present in PD patients. Other problematic motor symptoms of PD, such as resting tremor, gait problems and postural instability are thought to arise from other degenerated brainstem loci and thalamic circuitry.

Nigrostriatal dopaminergic neurones are highly complex structures, synapsing with target MSNs to activate complex downstream signaling cascades. These precise mechanisms are lost when A9 neurones degenerate in PD. In addition, further dynamic adaptations develop within the dopaminergic-medium spiny neurone (MSN) synapse with chronic L-dopa therapy. Deficiency of extracellular dopamine is thus only one of many consequences of striatal denervation in PD.

Our understanding of the normal striatal DA synapse has greatly evolved since the discovery of dopamine (DA) as a neurotransmitter [1]—we now appreciate the extensive arborisation of dopaminergic projections [2] and have greater knowledge of its robust pacemaking properties [3].

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There is greater understanding of pre-synaptic regulatory mechanisms and the complex post-synaptic modulatory effects of co-localising serotonergic and glutamatergic neurones in the caudate and putamen [4]. Dynamic pre and post-synaptic adaptations appear following midbrain DA neurones degeneration, contributing to the pathogenesis of distressing drug-induced side effects such as L-dopa induced dyskinesias (LID) [5].

Current pharmacological management options for PD focus solely on replacing deficient extracellular dopamine with dopamine agonists, monoamine oxidase-B inhibitors (MAO-B) or L-dopa, a dopamine precursor [6]. Although initially effective, an increasing proportion of L-dopa is converted by other AADC-containing structures such as blood vessels, serotonergic neurones and glial cells as the disease progresses, resulting in non-physiological pulsatile midbrain concentrations of DA with large swings in DA receptor occupancies. This approach to restoring a precise and complex synaptic system eventually leads to progressive reductions in clinical efficacy, as well as development of LIDs. We advocate for the current conceptual shift in treatment of PD from restoration of extracellular dopamine to replacement of lost striatal synapses. We illustrate how the optimal treatment of PD should aim to restore degenerated synapses, reproduction of physiological dopamine transmission at appropriate concentrations, timings, release patterns and locations, with normal regulatory mechanisms.

In this review, we describe the exquisitely regulated pre-synaptic mechanisms at the SNpc-MSN synapse, neurotransmitter release patterns and downstream modulatory signaling mechanisms within the MSN. We demonstrate how loss of these mechanisms and subsequent dynamic adaptations result in impaired DA modulation of other co-localising neurones within the basal ganglia. In addition, we discuss the feasibility of achieving these goals with neurotransplantation using foetal midbrain tissue, re-examine results from recent clinical foetal transplantation case studies and trials, identify key issues critical to the success of neurotransplantation as a therapeutic option for PD and discuss the optimal parameters for achieving maximal success with neurotransplantation.

Normal striatum

Arborisation

Nigrostriatal dopaminergic neurones form highly branched networks within their striatal targets. The full extent of this arborisation was elegantly demonstrated by Matsuda and colleagues, using rats injected with a sindbis virus-green fluorescent protein (GFP) anterograde neuronal tracer [2]. Nigrostriatal projections formed even wider and denser arborisation trees within the striatum than previously recognised: a single dopaminergic neurone innervated up to 5.7% of the neostriatum, while adjacent input dopaminergic neurones showed high degrees of overlapping with targets. It was calculated that a single post-synaptic MSN could be influenced by up to 194 dopaminergic inputs. As a result of this redundancy, clinical signs of PD only develop after up to 70% of NS neurones are lost [7]. However, little arborisation occurs outside the striatum, suggesting that DA neurones only branch extensively when within its natural target.

Autonomous pacemaker

SNpc dopaminergic neurones are autonomous pacemakers, generating 2–4 Hz action potentials without synaptic inputs. Pacemaking primarily relies on a mechanism involving calcium influx [8] via L-type Ca^{2+} channels [9], resulting in high concentrations of intracellular Ca^{2+} that cannot be immediately removed from cells and are instead sequestered [10]. Storage of Ca^{2+} around the endoplasmic reticulum and mitochondria increases the neurone's metabolic rate, resulting in the production of free radicals and reactive oxygen species. The combination of accumulated mitochondrial DNA damage and expensive metabolic mechanisms are thought to accelerate aging, leading to significantly higher rates of neuronal loss in SNpc compared to other areas of the brain [8]. Conservation of autonomous pacemaking despite such a high risk of cellular damage suggests a significant function in preserving steady striatal extracellular dopamine concentration necessary for normal network activity. Current L-dopa replacement therapies are unable to replicate this pacemaking property, with striatal autonomous firing achieved only with restoration of neuronal synapses.

Pre-synaptic autoregulation

A number of pre-synaptic mechanisms tightly regulate dopamine neurotransmission in the intact striatum. The dopamine transporter (DAT), which takes up and recycles released synaptic dopamine back into the pre-synaptic terminal, is known to be a key regulator in the duration of dopamine action, with different densities of DAT correlating to varying durations of dopamine effects [11]. Jones and colleagues demonstrated the significant roles other regulatory mechanisms play in controlling dopamine neurotransmission using wild-type and DAT knock-out (DATKO) mice [12]. Lack of functional DAT was confirmed with high-pressure liquid chromatography (HPLC), which showed intracellular DA in DATKO mice to be 5% of wild-type animals. Remarkably, extracellular dopamine

concentrations were found to be only five times greater in homozygous knock-outs compared to wild-type animals, despite a 300 fold increase in dopamine duration time in DAT mice. In compensation against reduced synaptic clearance, DATKO neurones reduced pre-synaptic DA release to standardized electrical stimulation compared to wild-type. At the same time, increased extracellular concentrations of dopamine breakdown products, such as 3-methoxytyramine (3MT) and homovallinic acid (HVA), were found in DAT KO animals, suggesting a compensatory increase in extracellular dopamine breakdown. Variations in release volumes and neurotransmitter breakdown thus also appear to contribute significantly to regulating extracellular dopamine.

Post-synaptic modulation of glutamatergic and serotonergic inputs

At the SNpc-MSN synapse, precise patterns of dopaminergic release modulate the transmission of glutamate and serotonin neurones inputting at the same synapse, mediated by a complex downstream signaling pathway involving closely regulated neurotransmitter volumes and temporal patterns. DA binding to D1 post-synaptic receptors increases the activity of adenyl cyclase via a positively coupled G-protein, increasing levels of cytosolic cAMP [13]. Subsequent activation of protein kinase A (PKA) leads to phosphorylation of a wide range of downstream signaling targets including dopamine and cAMP regulated phoshoprotein of 32 kDa (DARPP-32) [14]. The phosphorylation state of DARPP-32 subsequently acts as a switch that determines the modulatory effect of dopamine via immediate and short-term effects on AMPA and NMDA receptor gating and trafficking.

Firstly, specific temporal patterns of dopamine and glutamate transmission gate the activity of NMDA receptors, via differences in phosphorylation of the receptor's NR1 subunit. DA augments corticostriatal transmission only in the event of simultaneous MSN depolarization by sustained glutamate release [15]. In contrast, DA has no effect on corticostriatal transmission when there is no or only transient glutamatergic activity [16].

Secondly, surface expression of glutamatergic AMPA and NMDA receptors are determined by differential binding of dopamine to D1 or D2 receptors. D1 receptor activation increases expression of both AMPA and NMDA receptors via PKA activation [17, 18], while D2 activation leads to increased levels of DAG and protein kinase C (PKC) via a negatively coupled G-protein, resulting in the trafficking of AMPA receptors away from the synaptic membrane.

Thirdly, dopamine indirectly regulates the excitability of its MSN target by altering the rate of autonomous firing in cholinergic interneurones. DA binding to interneurone D2 receptors reduces calcium influx, lowering the rate of interneurone autonomous firing, reducing acetylcholine release and consequently increasing MSN post-synaptic excitability [15].

The complex spatial and temporal interplay between striatal dopamine, glutamate and acetylcholine neurones promotes desired, cortically driven motor actions while suppressing uncoordinated motor programmes. Co-activation of incompatible programmes is prevented by precise patterns of neurotransmission that pharmacological dopamine replacement therapies are unable to replicate.

Direct pathway supersensitivity with shift in downstream signalling pathway

In Parkinson's disease, a significant proportion of nigrostriatal dopaminergic neurones with high DAT density, projecting to the dorsolateral striatum, degenerate. Dynamic adaptations occur in the denervated PD striatum after loss of these A9 neurones, such as post-synaptic supersensitivity to D1 activation in the direct pathway [19], increased incidence of glutamate mediated synaptic events with loss of striatal synaptic plasticity [20] and impairment of pre-synaptic autoregulation [21], resulting in a dysfunctional SNpc-MSN synapse.

In the normal striatum, activation of D1 receptors in the direct pathway increases induction of immediate early genes (IEGs) such as transcription factor c-fos and zif 268 [22]. These effects precede changes in late-response genes, such as neurotransmitter and receptor translation. In the event of repeated D1 activation, a sustained, robust IEG response is not produced in the healthy striatum [23]. Instead, activation of IEGs diminishes, correlating with an increase in the normally low level of dynorphin in the dorsal striatum, blunting further induction of IEGs in the direct pathway and consequently preventing excessively robust responses to repetitive D1 receptor activation.

However, in the DA depleted striatum, repeated D1 receptors activation leads to continued increases in dynorphin levels without an adaptive blunting response [24]. MSNs switch to an alternative protein kinase pathway involving MAPKinase ERK1/2, which also eventually results in the activation of c-fos and other IEGs. The MAPKinase ERK1/2 pathway is normally activated only in the nucleus accumbens and appears to have no function in the healthy direct pathway [25]. Dysregulation of the MAPKinase ERK1/2 phosphorylation pathway is thus believed to be the mechanism behind post-synaptic supersensitivity in the denervated striatum. Replacement of striatal dopamine with direct D1 agonists or L-dopa restores deficient extracellular striatal dopamine concentrations but repeated administration continues to result in pulsatile activation of D1 receptors, repeatedly activating the

aberrant MAPKinase ERK1/2 pathway. The resultant supersensitive direct pathway has been postulated to contribute towards L-dopa dyskinesias (LIDs) pathogenesis [19].

Impaired synaptic plasticity

Striatal denervation results in impairment of normal striatal synaptic plasticity. In the normal striatum, depolarization of the MSN post-synaptic terminal by D1 activation relieves voltage dependent NMDA block, inducing long-term potentiation in the synapse [26]. The specific requirement of D1 and NMDA receptors for LTP induction has been demonstrated by pharmacological and genetic knock out experiments, which showed that impairment of D1 or NMDA receptors both result in lack of LTP induction [27]. D2 receptors appear to have an opposite role in MSN LTP, with enhanced LTP induced with D2 pharmacological antagonist block or D2 gene knock out [28]. In addition, long term depression (LTD) is thought to require the simultaneous activation of D1, D2, AMPA and metabotropic glutamate receptors, resulting in a rise in intracellular calcium and activation of calcium dependent protein kinases, producing striatal LTD [29]. Considering the central roles of both dopamine and glutamate transmission in MSN plasticity, nigrostriatal denervation and changes in glutamatergic transmission have been implicated in abnormal MSN synaptic plasticity development.

Functional and anatomical changes

Functional and anatomical changes are also observed in response to reductions in extracellular DA concentrations. Co-localising glutamatergic neurones compensate by increasing firing rates [30] while glutamatergic inputs form new synaptic terminals with MSNs [31]. With further denervation in PD, progressively lower densities of presynaptic autoregulatory mechanisms results in reduced synaptic dopamine recycling. D1 receptors are internalized in the direct pathway while the density of spines on target MSN dendrites decrease, as has been observed in 6OHDA rats and post-mortem analyses of human PD patients.

There is also change in the expression of late genes such as neuropeptide and dopamine receptors in the direct and indirect pathways. Reduction in D1 receptor activation leads to reduced substance P, dynorphin and D1 receptor expression. Contrastingly, reduction in D2 receptor activation decreases G-protein coupled inhibition, leading to increased enkephalin and D2 receptor expression [19]. These gene expression changes lead to a net increase in activity of the indirect pathway while decreasing the influence of the direct pathway, producing a simplistic explanation for the bradykinetic features of PD. Although dopamine replacement therapies can be successful in restoring extracellular DA concentrations, they are unable to reverse functional and anatomical changes in the denervated striatum.

Denervated striatum in the PD patient

Pulsatile dopamine release

The eventual use of L-dopa in most PD patients results in a non-physiological pulsatile supply of DA into the striatum. However, with progressive loss of native dopaminergic neurones, greater proportions of L-dopa are converted into dopamine by other AADC containing structures, such as blood vessels, glial cells and remaining serotonergic neurones. Their lack of autoregulatory mechanisms produces non-physiological cycles of pulsatile dopamine release, leading to large swings in dopamine receptor occupancies [32].

Dysregulation of L-dopa conversion to DA by serotenergic afferents lead to release of DA as a false transmitter in inappropriate striatal locations, which has been associated with L-dopa dyskinesia development [33]. Removal of serotonergic afferents and blocking of serotonergic activity by $5HT_{1A}$ and $5HT_{1B}$ agonists have been shown to dampen LIDs in rat models [34] and human patients [35]. Against a background of dysfunctional DA-MSN synapses and aberrant conversion by unwanted structures, L-dopa treatment produces progressively reduced clinical benefits as a result of increasing disability from worsening side effects such as LIDs, despite sometimes persistent improvements in UPDRS motor scores.

Chronic denervation leads to dysfunctional dopaminergic phosphorylation pathways, causing impaired long term depotentiation

While impaired synaptic plasticity is partially corrected with restoration of LTP, chronic L-dopa treatment leads to dysfunctional phosphorylation of downstream signaling pathway, causing continued impairment of long term depotentiation that cannot be reversed by dopamine replacement therapies alone [20].

In the normal striatum, synaptic potentiation is maintained and reduced by a post-synaptic phosphorylation pathway balanced by protein kinase A (PKA) and cyclin-dependent kinase-5 (CDK5) respectively. Binding of dopamine to D1 receptors lead to increased levels of cAMP by adenylate cyclase, enhancing PKA activity, leading to phosphorylation of DARRP32 and subsequent inhibition of protein phosphatase 1(PP1) [36]. Interactions between PP1 and post-synaptic density-95 (PSD-95) proteins such as

calmodulin-dependent protein kinase II (CAMK-II) modulate the state of potentiation at NMDA receptor subunits, controlling the efficacy of the corticostriatal glutamatergic inputs [37].

The state of DARPP32 phosphorylation is the key controlling step in the modulation of synaptic potentiation in the normal striatum. Loss of long term depotentiation at the corticostriatal glutamatergic synapse, as a result of dysfunctional DA downstream phosphorylation, is thought to result in an impaired ability to "forget" unwanted movements[38], postulated to contribute towards involuntary movements such as LID following chronic L-dopa [20]. Experimental evidence for this hypothesis has been observed in 6OHDA rats kept in a chronic L-dopa therapy paradigm. Those who did not develop LIDs were found to be able to depotentiate the corticostriatal synapse following low frequency activation protocols while those exhibiting LIDs were unable to depotentiate [28]. In addition, the striata of dyskinetic rats were found to contain abnormally high levels of phosphorylated DARPP32, further implicating dysfunctional DA phosphorylation in LID pathogenesis [28]. While L-dopa treatment is effective in restoring long-term potentiation, chronic use impairs synaptic depotentiation, resulting in dyskinesias observed with chronic L-dopa therapies.

Expression of GABA-A and PPE-A/B

Long-term pulsatile dopamine replacement against a background of impaired pre-synaptic mechanisms and dysfunctional post-synaptic plasticity leads to increased MSN expression preproenkephalin (PPE) and enhanced effects of GABA, consequently contributing to development of LIDs. Increased PPE activity in MSNs after chronic L-dopa therapy has been demonstrated in animal models, human post-mortem examinations, imaging studies and pharmacological trials. High levels of PPE mRNA are found in striata of MPTP monkeys [39] and human PD patients [40]. Imaging of PD patients with LID using PET shows reduced diprenorphine binding to opioid receptors, indirectly demonstrating increased neuropeptide activity [41]. Pharmacologically, use of mu and delta opioid receptor antagonists appear to reduce LID symptoms in MPTP marmosets while post-mortems of PD patients on dopaminergic replacement therapies that do not induce LID demonstrated normal PPEmRNA transcripts [42]. However, there is as yet no direct evidence that increases in PPE transcription equates to an increase in PPE release. In addition to increased PPE activity, chronic L-dopa use leads to upregulation of GABA-A receptors in MSN target neurones [43]. As a result, chronic L-dopa therapy against a background of dysfunctional post-synaptic mechanisms is thought to lead to supersensitive GABAergic inputs in the direct pathway, increasing the degree of inhibition on globus pallidus internus (GPi), reducing inhibition on the thalamus and producing subsequent dysregulation of movements. Imbalance of the direct and indirect pathways is thus thought to cause development of LIDs after treatment with chronic L-dopa therapy alone.

Synapses not dopamine

The potential benefits of restoring DA synapses were recently demonstrated by Vinuela and colleagues in a mouse PD model, implanted with cell suspensions of either wild-type foetal mouse midbrain or donor tissue genetically knocked out for dopamine transporter (DATKO) [44]. Significant motor improvements without off-medication dyskinesias were observed in all transplanted animals, even in mice grafted with DATKO tissue, despite very high DA levels in the synaptic cleft. Nigrostriatal DA synapses, even if not fully functional, can provide functional benefits without inducing dyskinesias. Notably, greater functional improvements were observed in animals receiving wild-type transplants containing greater numbers of DAT and bearing closer resemblance to the A9 neurones preferentially lost in PD.

Is neurotransplantation in PD feasible?

Although animal foetal midbrain tissues have been shown to survive following transplantation into host rodent brains, the ability of grafted neurones to grow to appropriate targets was only demonstrated by experiments in the early 1990s. While Wictorin and colleagues showed the growth distance possible with transplanted axons [45], Deacon and Isacson conclusively demonstrated the remarkable precision, specificity and distinct growth patterns that grafted porcine neurones can reach their rat axonal targets [46, 47]. The adult mammalian CNS appears to contain sufficient information, in the form of diffusible or substrate-bound guidance molecules and factor gradients, to guide axons to their appropriate targets.

Are functional improvements possible with PD neurotransplantation?

The best examples of the potential benefits of PD neurotransplantation are two patients recently reported by Politis and colleagues [35]. Previously suffering from severe motor complications of L-dopa therapy, including diphasic and L-dopa induced dyskinesias, weaning off phenomena but no off-period dyskinesias, the two patients received foetal VM tissue transplants into bilateral putamina 16 years ago, and bilateral caudate nuclei and putamina 13 years ago respectively.

The first patient demonstrated only moderate improvements in PD symptoms and dyskinesias for 3 years after surgery, with shorter and fewer off-periods, as well as a reductions in on-period dyskinesias. However, he began to demonstrate significant improvements in PD symptoms from the fourth post-operative year onwards. Sixteen years after grafting, the patient requires no dopaminergic medications and suffers from no off-periods, with UPDRS part III (motor) scores reduced from 38 to 13, 16 years post-operatively.

The second patient underwent a similar delay before significant motor improvements were observed. There were no changes in PD symptoms for 3 years post-operatively. The patient's UPDRS part III scores actually increased from 22 pre-operatively to 26 in the first year after transplantation. However, gradual improvements began from the fourth post-operative year onwards, with all dopaminergic medications being stopped from 5 years post op. Thirteen years after transplantation, UPDRS part III scores have decreased from 22 pre-operatively to 7, with no off-period dyskinesias.

However, both patients have experienced involuntary movements known as graft-induced dyskinesias (GIDs), involving the trunk, orofacial muscles, upper and lower extremities. They have become disabling and are uncontrolled by amantadine. Significantly, these dyskinesias were markedly diminished with dampening of serotonergic transmission with a 5HT-1A agonist. Such significant new insight has important implications for future midbrain grafts, demonstrating that GIDs are potentially avoidable and treatable, minimising the obstacle that GIDs pose towards significant therapeutic potential of neurotransplantation in PD patients.

Intriguingly, other earlier human foetal grafting trials produced significantly less impressive outcomes. An early study by Freed and colleagues involving seven patients produced beneficial but far less substantial improvements [48]. Despite all patients reporting improvements in activities of daily living (ADL) scores, reductions in motor symptoms and diminished incidence of dyskinesias, only five of seven patients demonstrated any improvements in UPDRS scores at 6 month follow up. Two patients showed no motor improvements at all. Why did these studies produce such variable functional benefits? There appeared to be significant differences in grafting paradigms between these studies, such as graft composition, immunosuppresion, type of grafts, cell preparation methods, graft location, surgical technique and patient selection. But, perhaps most notably, the potential for functional improvements were demonstrated despite the lack of the most optimal grafting parameters.

What are the important parameters in foetal grafting?

Graft composition

Restoration of the mesencephalic A9 dopaminergic neurones particularly vulnerable to degeneration in PD is central towards graft efficacy. However, recently transplanted grafts are thought to contain no more than 10% A9 neurones [49]. Theoretically, the higher numbers of presynaptic dopamine autoreceptors and dopamine transporters in A9 neurones make them more capable of controlling synaptic DA transmission than adjacent A10 [50]. In addition, A9 neurones are also intrinsically more able to form extensive networks within the dorsolateral striatum than A10 neurones [51].

Grealish and colleagues elegantly demonstrated the importance of A9 neurone restoration in providing maximal motor benefits [52]. Transplanting PD model rodents with either a donor graft composed of A9 and A10 neurones or A10 neurones alone, animals grafted with both types of DA neurones performed far superiorly in motor tasks compared to rats receiving grafts selectively lacking in A9 neurones. Consistent with PET imaging evidence from grafted PD patients, A9 neurone innervation of the dorsal striatum is thus crucial for optimal functional improvements after grafting.

A10 neurones are also thought to play a supportive role in graft efficacy. In rodent models of PD, animals with intact A10 targets such as the ventral striatum and nucleus accumbens demonstrated greater motor improvements after grafting with foetal midbrain tissue compared to rodents with extensive midbrain lesions [53]. However, their exact function remains to be addressed in future studies. Nonetheless, considering the central role of A9 neurones in producing significant graft-induced motor improvements, the aim of PD neurotransplantation should be the production and transplantation of neuronal grafts with high densities of A9 neurones, via optimized cell preparation and cell-sorting using standardized stem cell production techniques.

Variable compositions of A9 and A10 neurones, and in particular, inclusion of serotonergic neurones, are likely to contribute towards development of GIDs. Observed in 15% of patients in the first large, randomized, double-blinded trial in 2001 [54], GIDs were previously thought to result from excess DA release from graft outgrowth and differences in DA graft volume release. However, neither excess in graft DA uptake or graft DA release were demonstrated in PET imaging studies using ^{18}F-dopa uptake and ^{11}C raclopride binding respectively. There was also no correlation between DA release on functional imaging and the severity of off-dyskinesias [35].

Instead, excess serotonergic innervation was shown using PET imaging in two patients with disabling GIDs [35]. Binding of [11] C-DASB to 5HT transporters, a marker of serotonin neurone density, was increased in grafted areas compared to a concurrent decrease in non-grafted PD striatum, with a greater increase in serotonergic innervation correlated to worse GID scores. Although whether the two are produced by the same mechanism is unknown, the increased serotonin-dopamine terminal ratio observed in GID patients is similar to that observed in animal models of LID. Significantly, Politis and colleagues demonstrated that GIDs could be diminished with administration of a $5HT_{1A}$ agonist, inhibiting serotonergic activity by reducing false release of DA from 5HT terminals [35]. It is hypothesized that a net increase in serotonergic neurones and a net decrease in DAT density in the grafted tissue, placed into an area deficient of DA autoregulation mechanisms following striatal denervation, would result in large swings in extracellular dopamine concentrations, causing development of GIDs. These recent findings have significant implications for future grafts, with increased emphasis on reducing the proportion of serotonergic neurones in order to minimize incidence of GIDs. Politis and colleagues have suggested the use of fresh midbrain grafts [55], as long-term storage is thought to increase the proportion of serotonergic neurones within the graft, along with careful dissection of foetal VM tissue [56]. The association between serotonergic neurones and GID development implicate the need for improved cell sorting techniques when producing VM grafts from stem cells, ideally aiming for increased composition of A9 and fewer serotonergic neurones.

Local graft inflammation

The degree of localized inflammation around the grafted tissue is thought to be an important factor in the success of foetal neurotransplantation. Graft efficacy in reducing motor symptoms, development of PD-like pathology within the grafted tissue and incidences of GIDs appear to vary from study to study with different regimes of immunosuppression and grafting with solid tissues or cell suspension grafts, with solid tissues associated with an increase in local inflammation.

In two double-blinded, placebo-controlled PD foetal transplantation trials that failed to reach their primary endpoint, Freed in 2001 [54] and Olanow in 2003 [57] used no immunosuppression and only 6 months of immunosuppression respectively. In the latter trial, graft efficacy appeared to decrease abruptly following withdrawal of immunosuppression 6 months after transplantation. In contrast, when immunosuppression was stopped in transplanted patients 29 months after their final graft, Piccini and colleagues observed no reductions in UDPRS motor scores or ^{18}F-dopa uptake [58]. A period of sustained local inflammation dampening around the graft appears to be required for initiation and maintenance of graft efficacy in improving PD motor symptoms.

The transplantation of solid, non-dissociated tissues has been associated with an increase in localized inflammation and has been hypothesised to result in reduced graft efficacy, and leads to PD-like pathology development. Post-mortem analysis of one transplanted patient from Freed's cohort, in which solid, non-dissociated tissue was transplanted instead of cell suspensions, found numerous Lewy Body like inclusions and Lewy neurites within the graft and host striatum, as well as marked microglia activation around the graft [59]. In contrast, a separate Canadian study found no morphological evidence of neurodegeneration at post-mortem in the grafts of five patients receiving dissociated cell suspension grafts, reporting no alpha-synuclein, ubiquitin or lipofuscin inclusions, and only minimal microglia activation in the adjacent tissues [60]. The association of PD-like graft pathology with increased adjacent microglial activation in non-dissociated tissue grafts has been suggested to be mediated by donor blood vessels, resulting in greater expression of donor major histocompatibility complexes class I (MHC I) than with cell suspension grafts, in which host angiogenic process predominate instead [61]. Processes such as oxidative stress and accelerated aging [62], graft and adjacent tissue inflammation are hypothesized to produce PD histological markers within transplanted neurons, compromising graft-host interactions required for tissue integration, reducing clinical efficacy [63].

Local graft inflammation has also been implicated in the pathogenesis of GIDs. The first large-scale foetal transplantation trial [54], which used no immunosuppression, reported GID incidences of up to 15%. In addition, significant increases in dyskinesia scores were reported in Piccini's cohort when immunosuppresion was removed 29 months after grafting, despite no change in UPDRS motor scores in ^{18}F-dopa uptake on PET imaging [58]. Removal of immunosuppression allows increased graft growth, including growth of serotonergic and non-DA cells implicated with GIDs. However, the association between graft inflammation and GID pathogenesis is somewhat dissatisfactorily incomplete: for example, a number of grafted patients in Piccini's cohort developed GIDs prior to the withdrawal of immunosuppression. Also, GIDs appear to persist in grafted patients years after transplantation, with no reports of GIDs being more severe immediately after surgery, when inflammation is expected to be greatest. A central role for local inflammation in GID pathogenesis would instead anticipate a gradual reduction in dyskinesia scores following transplantation.

Graft location, cell preparation and surgical technique

While transplanted neurones have the intrinsic ability to grow towards their appropriate target, graft positioning determines the ease of target finding and the distance the neuron is required to travel. Grafted DA neurones appear to reach their SN targets more easily when placed in the putamen of AFG monkeys compared to transplantation in the caudate [64]. Imprecise graft placement outside the intended caudate and putaminal targets could also result in the graft receiving a lack of normal local substantia nigral growth support factors, resulting in reduced long-term graft efficacy.

The precision of foetal tissue dissection, the length of cell hibernation and the choice of growth factors used such as GDNF are also important parameters in foetal transplantation [65]. In addition, the approach utilised by the surgeon is a significant factor in determining clinical efficacy, with optimal transplant techniques using the lowest numbers of needle passes, hence triggering the minimal amounts of local inflammation while placing cell suspension grafts accurately at multiple foci.

Patient selection

The degree of denervation in areas surrounding the graft appears to be a significant determinant of subsequent graft efficacy. Evidence from functional imaging studies have shown that while DA uptake is increased in grafted striatal areas, DA uptake continues to fall in the Raphe nucleus and substantia nigra, indicating that degeneration and regeneration continue simultaneously after grafting [58]. Previous foetal grafting studies in 6OHDA lesioned rats demonstrated greater functional improvement in animals with only caudate and putaminal lesions compared to those with complete mesencephalic loss [53].

In human patients, Piccini and colleagues demonstrated the correlation between lower levels of pre- and post-operative ^{18}F-dopa uptake in the ventral striatum and poorer graft-induced clinical improvements [58]. Reduction in DA uptake, a marker of more extensive denervation in the ventral striatum, appears to be a predictive factor in determining the future graft-induced therapeutic benefit. In addition, retrospective analysis by Ma and colleagues showed that the greatest functional benefits occur in patients with the highest pre-operative flourodopa PET uptake, while patients with extensive nigrostriatal degeneration received fewer benefits from transplantation [66]. The greatest benefits with neurotransplantation are observed in PD patients with sufficiently advanced symptoms but without extensive nigrostriatal degeneration, perhaps before significant disease involvement outside the basal ganglia or significant development of side effects such as dyskinesias. It appears that sufficient sparing of host DA systems innervating the ventral striatum and cortex are required for restored putaminal DA synapses to result in significant clinical benefits. There is perhaps an additional early role for PD grafting, offering a theoretical protective element via the in vivo production of trophic factors, slowing or halting the ongoing disease process. Accurate clinical identification of this optimal period for transplantation would be difficult but experimental paradigms using pre-operative ^{18}F-dopa uptake PET imaging has proved successful and could be used in the clinical setting to identify PD patients most likely to benefit from neurotransplantation [58].

Do transplanted foetal grafts survive?

Post-mortem examinations on PD patients who had received foetal transplants demonstrated long-term survival of VM grafts, with good graft integration and extensive neuritic outgrowths without host striatal tissue displacement. Mendez and colleagues found large numbers of neurones staining positive for tyrosine hydroxylase (TH+), a marker for DA neurones, in the grafts of two PD patients dying of unrelated causes 3.5 and 4.5 years after transplantation, correlating in both patients with significant functional benefits such as 50% reductions in UPDRS scores, reduced incidence of dyskinesias and increased striatal fluorodopa uptake on PET imaging [67]. Regarding the maximum lifespan of foetal grafts, Li and Brundin reported a surviving 16 year old graft containing large numbers of DA neurones at post-mortem, interestingly containing similar cell numbers to 18 month to 4 year old grafts transplanted using similar parameters [68]. Earlier fluorodopa and raclopride uptake PET imaging studies on these patients during life had found DA synthesis and release to have returned to normal levels ten years after transplantation [69]. Maintenance of good graft function is possible for up to a decade after transplantation, with graft survival possible for at least 16 years.

Do foetal grafts develop PD?

Although there are convincing reports of long term graft survival, evidence for graft pathology has so far been ambiguous. While some post-mortem case studies have reported extensive Lewy bodies and Lewy neurites, others have found no or minimal amounts of PD histopathology. Post-mortem of a 14 year old bilateral solid foetal graft found significant numbers of cytoplasmic, aggregated and neuritic alpha-synuclein [70]. Although significant functional benefits persisted for 11 years, these improvements deteriorated during the final 4 years of life, questioning the presence of PD pathology development within previously

functioning grafts. Similarly, Li and Brundin reported significant amounts of alpha-synuclein in unilateral solid foetal grafts, with 40 and 80% of TH+ neurones found to contain detectable levels of alpha synuclein in 12 and 16 year old grafts respectively. Lewy bodies and Lewy neurites, morphologically and immunologically indistinguishable from those found in the adjacent host striatum, were observed in the oldest grafted neurones [68].

On the other hand, Mendez and colleagues reported only limited PD pathology in five dissociated, cell suspension grafts up to 14 years old [60]. The appearance of PD pathology in grafted tissue has been hypothesized to result from localised inflammation leading to oxidative stress, causing accelerated aging and consequent graft PD pathology [69]. Both grafts found to contain significant PD pathology were associated with significantly higher levels of microglial activation compared to the host striatum. Although a parallel hypothesis has suggested graft PD pathology to be a result of host α-synuclein seeding, we believe this to likely be limited and a minor cellular mechanism. As Li and Brundin noted, the majority of grafted neurones appeared functionally unimpaired, even in grafts containing significant PD-like pathology [68]. Graft PD-like pathology is thus unlikely to significantly affect function, as demonstrated by clinical benefits observed up to 17 years after transplantation despite the use of suboptimal parameters [35].

Why did double-blinded placebo-controlled trials not demonstrate functional improvements?

With the recent demonstration of continued graft-induced motor improvements for up to 17 years after transplantation, with grafted patients not requiring any PD medications [35], it is intriguing that the original two double blinded, placebo-controlled trials failed to demonstrate clinical improvements. However, longer-term follow-up by Ma and colleagues of patients from the first randomized trial in 2001 demonstrated that foetal transplantation did produce significant motor benefits across all age groups of transplanted patients [66].

In the first trial led by Freed and Fahn, 40 PD patients were randomized to either bilateral solid non-dissociated foetal tissue grafting or sham surgery with no immunosuppression regime [54]. VM putaminal foetal tissues were obtained less than 4 weeks prior to transplantation and were incubated in a culture medium containing 5% human placental serum. Tissue from the same foetus was transplanted stereotactically along two needle tracks for each side of brain being grafted. The non-dissociated tissue was extruded through a sterile glass device as 200 micrometer strands. Patients randomized to sham surgery received identical drill holes within their skulls following stereotactic ring fixation but without penetration of dura. All patients were assessed before surgery and at 1 year after transplantation, comparing parkinsonian motor symptoms, levels of intrastriatal DA synthesis and release and presence of side effects. While seventeen of the twenty patients transplanted patients demonstrated increases in intrastriatal DA synthesis and release on PET imaging, significant UPDRS score improvements were only observed in the younger cohort, with no patient over the age of 60 showing any significant motor benefits at 1 year. Worryingly, 15% of transplanted patients developed GIDs.

In a second trial led by Olanow in 2003, 24 PD patients were randomized to bilateral solid nigral grafts or sham surgery [57]. Tissue was obtained less than 2 days prior to operation from electively aborted foetuses without undergoing further cell culture. Transplanted tissue was deposited into eight needle tracts on each side of the brain, with tissue from up to four foetuses used per grafted side. All patients were treated with cyclosporine 2 weeks prior to the operation, with the immunosuppressive dose reduced 2 weeks post-operatively and 6 months after surgery. Patients were assessed at 2 years after transplantation. While significant increases in fluorodopa and raclopride uptake were again found PET imaging, these improvements in DA metabolism did not translate into functional benefits, with no significant differences found before and after surgery as measured on UPDRS scores. In addition, a far larger proportion of grafted patients (56%) developed GIDs following transplantation.

These initial findings suggested that while effective at improving intrastriatal DA synthesis and release, foetal transplantation does not produce significant functional benefits. This preliminary conclusion contradicted the huge clinical potential demonstrated by earlier, smaller open-labeled case studies. However, after the original clinical endpoint of 1 year, 14 of 20 patients from Freed and Fahn's 2001 trial originally randomized to sham surgery also received bilateral solid foetal VM grafts, increasing the total number of grafted patients from 19 to 33. Of these 33 patients, 18 were followed up for 2 years after transplantation, while the remaining 15 were observed for up to 4 years after surgery. When assessed at the 2 year time point, all grafted patients demonstrated significant motor improvements, which were sustained to the 4 year assessment point in patients receiving extended follow up, with benefits not limited to younger patients. PET imaging demonstrated an increase in posterior putaminal ^{18}F-dopa uptake continuing to the 4 year time point, with the greatest increases during the first 2 years after transplantation, correlating with functional improvements as measured by UPDRS scores [66].

These findings strongly suggest a period of graft maturation required for functional benefits to develop,

concurring with results from previous cases studies in which no motor improvements were demonstrated for the first 6 months following transplantation. PET imaging also implicated progressively increasing graft function for at least 4 years after grafting. It is very likely that both the 2001 and 2003 clinical trials, with primary endpoints designated at 1 and 2 years respectively, assessed for motor improvements too early. Re-evaluation of results suggests a longer period of graft maturation was required for older patients, highlighting consideration for future clinical trial endpoint designation.

However, it should be noted that only a proportion of transplanted patients were followed up to 2 and 4 years. Of the 33 original patients, 29 were followed to 2 years post-operatively and only 14 to 4 years, with more older patients lost to follow-up than younger patients, subjecting results to a possible graft efficacy bias in view of earlier conclusions of increased graft efficacy in younger patients. In addition, while patients over 60 demonstrated continuing decreases in UPDRS motor scores from 2 to 4 years post-operatively, UPDRS scores actually increased between 2 and 4 years for the under 60 cohort, with a least five patients in this group developing significant GIDs. Although implying the presence of long-term clinical improvements with foetal transplantation, this long-term follow up study should nonetheless be interpreted with caution.

Perhaps most significantly, functional benefits were demonstrated without the use of most optimal transplantation paradigms. No immunosupression was used in the first trial and only 6 months post-operatively in the second. Secondly, transplanted patients were not selected with pre-operative PET to detect the extent of ventral striatal denervation. Both trials were open to patients of all disease severities. Thirdly, both trials transplanted patients with fragmented solid non-dissociated pieces of foetal midbrain instead of cell suspension grafts. It would not have been possible to precisely dissect away all blood vessel-containing membranes, significantly increasing the numbers of graft MHC-I complexes and consequent microglial activation. Subsequent limitations of graft integration with their posterior putaminal targets, as well as possible graft maturation restriction, could explain the lack of further functional benefits between 2 and 4 years after transplantation in the 2001 trial.

The use of solid non-dissociated foetal tissues could contribute to the high incidence of GIDs in both trials. While demonstrating the greatest functional improvements, the youngest patients were also at highest risk of developing GIDs, suggesting a correlation between ability to mount an inflammatory response with the GIDs incidence. Lastly, grafted tissue was likely to have contained a mixture of cell types in addition to A9 DA neurones. Serotonergic neurones, implicated in development of GIDs, and A10 DA neurones, which are hypothetically less able to control physiological DA transmission due to fewer autoregulatory mechanisms, would also have been transplanted.

Are neurotransplants a reasonable restorative treatment for PD?

Although foetal transplantation cannot perfectly restore degenerated midbrain DA synapses or ultimately provide the perfect cure for PD, we argue that these imperfections do not detract from neurotransplantation's therapeutic prospect as a potential treatment option in PD.

Firstly, although foetal grafts almost never restore intrastriatal DA to normal concentrations, the relatively small increase could be sufficient to raise DA concentrations beyond a threshold, above which PD motor symptoms do not appear despite substantial DA depletion. Likely to be a result of the extensive midbrain DA neuronal arborisation and large numbers of synaptic connections, PD symptoms only manifest after an estimated 70% of functional DA synapses are lost and intrastriatal DA concentrations have been reduced to less than 40% of its normal levels [7, 63]. Allowing for a steady 6% annual decline in native striatal function and stochastic protein aggregate-induced graft functional impairment, it has been calculated that a well-functioning graft can provide symptomatic improvements for in excess of 20 years [63]. Neurotransplantation can thus provide PD symptomatic relief without fully restoring DA concentrations.

Secondly, although transplanted neurones do not form synapses at the exact anatomical locations as native DA neurones, perfect anatomical reconstructions are not essential for producing functional benefits. Grafted DA neurones appear not to necessarily favour synapses at MSN spines like native nigrostriatal projections, but also synapse at dendritic shafts. However, it appears the replacement of pre- and post-synaptic DA regulatory mechanisms and autonomous pacemaking are more important factors in producing functional benefits.

Thirdly, despite our insight for motor symptoms arising from nigrostriatal degeneration, PD lesions affecting locations outside the basal ganglia are less well described but nonetheless need to be addressed as part of a complete management plan for PD patients. It is known that cerebellar inputs [71] and brainstem nuclei, including the dorsal motor nuclei of the vagus (dmX) [72], also degenerate in PD. Lesions in the pedunculopontine nuclei (PPN) are thought to contribute towards axial symptoms respectively [73]. In addition, significant reductions in cortical and striatal cholinergic transmission have been demonstrated in PD patients without dementia, PD patients with dementia

(PDD) and patients with dementia with Lewy Body (DLB), as demonstrated by reductions in markers of cholinergic transmission such as choline acetyltransferase (ChAT), acetylcholinesterase (AChE) and nicotinic and muscarinic cholinergic receptors, resulting in symptoms such as psychosis and memory impairment [74]. Degeneration of postganglionic autonomic and other peripheral nerves have been well reported, leading to gastrointestinal dysmotility [75], cardiac dysrhythmias, micturition symptoms, dysarthria and dysphagia, as well as widespread multi-organ distribution of alpha-synuclein histopathology on post-mortem [76].

However, neurotransplantation remains amongst the best in a limited repertoire of options for symptoms originating from outside the basal ganglia. Although promising results have been reported from PPN deep brain stimulation (DBS) in a cohort of six patients, with major improvements in gait and posture in one PD patient at 1 year follow up and moderate improvements in four others [77], the procedure is still not widely performed. Meanwhile, L-dopa therapy is ineffective for treating gait and axial symptoms of PD whilst producing distressing side effects. With the development of novel biotechnology, lesions outside the basal ganglia could perhaps be approached similarly to degenerated nigrostriatal projections, with transplantation of neurones in GI and cardiac autonomic circuits, or restoration of degenerated forebrain cholinergic neurones, plausible therapeutic possibilities for the future.

A European research council supported clinical trial due to begin in 2011 will aim to define optimal parameters for maximal functional benefits with foetal transplantation in human PD patients, characterizing the most responsive patient group as well as recommending optimal graft types and cell preparation paradigms. Future clinical trials can subsequently define the maximal dose-efficacy relationship provided by cell replacement in PD. Following rodent PD experiments demonstrating the significance of neuronal subtype specificity in determining the degree of functional benefits, with greater proportion of putaminal- projecting A9 DA neurones resulting in greatest motor recovery [50], the goal of PD cell replacement remains the generation of uniform A9 DA neuronal grafts, ultimately from induced pluripotent stem cells (iPSC) [50]. In order to demonstrate the potential of iPSC derived mDA neurones as a suitable donor source, it will be appropriate in the near future to begin proof-of-principle grafting experiments in animal models including non-human primates, followed by small human empirical studies. Potentially, the next future step for PD neurotransplantation may involve production of iPSCs of A9 neurones, increasing clinical efficacy without the supply and ethical issues of foetal grafts.

Conflict of interest None.

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