Endogenous *versus* exogenous cell replacement for Parkinson's disease: where are we at and where are we going?

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From the Contents

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Abstract

Parkinson's disease is the second most common neurodegenerative disease and has currently no effective treatment, one that would be able to stop or reverse the loss of dopaminergic neurons in the substantia nigra pars compacta. In addition, Parkinson's disease diagnosis is typically done when a significant percentage of the dopaminergic neurons is already lost. In neurodegenerative disorders, some therapeutic strategies could be effective only at inhibiting further degeneration; on the other hand, cell replacement therapies aim at replacing lost neurons, an approach that would be ideal for the treatment of Parkinson's disease. Many cell replacement therapies have been tested since the 1970s in the field of Parkinson's disease; however, there are still significant limitations prohibiting a successful clinical application. From the first fetal midbrain intrastriatal graft to the most recent conversion of astrocytes into dopaminergic neurons, we have gained equally, significant insights and questions still looking for an answer. This review aims to summarize the main milestones in cell replacement approaches against Parkinson's disease. By focusing on achievements and failures, as well as on the additional research steps needed, we aim to provide perspective on how future cell replacement therapies treats Parkinson's disease.

Key Words: endogenous; neurodegenerative disease; neurogenesis; neurotrophic factors; Parkinson's disease; stem cells; transdifferentiation; transplantations

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease that affects approximately 9.4 million people worldwide, consisting a significant socioeconomic problem (Maserejian, 2020). In the present, there is no long-term effective treatment able to stop or reverse the neurodegenerative process.

During the last decade, there have been many preclinical advances in cell replacement therapeutic strategies (CRTs) for the nervous system, which are evaluated for the treatment of neurodegenerative diseases and neural injury. Although a lot of promising preclinical data have been published, to date there are many limitations regarding the safety and efficacy of CRTs, prohibiting a successful clinical application (De Gioia et al., 2020; **Figure 1**).

There are 3 main approaches for cell replacement in the degenerated brain: exogenous transplantations of allogeneic or autologous stem cells, direct *in vivo* reprogramming of local cell populations towards neurons and manipulation of endogenous adult neurogenesis in the areas of interest, in order to enhance the ability of the brain to repair itself (**Figure 1**).

The first preclinical efforts for stem-cell based dopamine substitution against PD date back to the 1970's and led to several clinical trials in the 00's. Although those trials exhibited mixed results regarding efficiency, they proved that the rationale for a CRT strategy against PD is valid, since in many cases, significant functional improvement was observed (Guo et al., 2021). This is further supported by far more recent preclinical findings that either support that graft- or direct reprogramming-based CRTs are highly effective in restoring the nigrostriatal pathway and its function (Gaillard et al., 2009; Qian et al., 2020) or suggest that the adult ventral midbrain, and, more specifically, the SNpc itself is highly permissive for neurogenesis (in contrast to other areas of the adult brain) (Maya-Espinosa et al., 2015; Collazo-Navarrete et al., 2019). This implies that, with correct handling, PD could be ideal for an effective CRT. The aforementioned strategies, with their respective advantages and drawbacks will be discussed in detail throughout the following chapters.

Search Strategy and Selection Criteria

We used the "PubMed" and "Google Scholar" search engines and different combinations of keywords: "Parkinson" AND "transplantation", "Parkinson" AND "cell replacement therapy", "Parkinson" AND "Adult neurogenesis". The search results were sorted by relevance and the first 100 hits were checked. Additional searches using more specific keywords were also performed as needed (e.g., "neurotrophins", "Parkinson" and "transplantations", or "epigenetics", "neuronal" and "direct conversion"). The papers included in the manuscript were published between 1981 and 2021.

Cell Transplantations – Insights from Clinical and Preclinical Studies

Exogenous CRT approaches, using various types of grafts, have been investigated for the treatment of PD since the late 1970's (Björklund et al., 1981, 1982; Brundin et al., 1985). Due to the high variability of results, although cell grafts showed a lot of promise since day one, 40 years later, a widescale clinical approach is still missing. This heterogeneity is probably a result of the different protocols and PD models used in those studies. Until today, a lot of questions remain open regarding the optimal methodological parameters for transplantations [extensively reviewed elsewhere (Barbuti et al., 2021; Xiao et al., 2021)]; for example, the ideal cell type for transplantations [e.g. fetal ventral midbrain tissue, human embryonic stem cells (hESCs), induced pluripotent stem cells (iPSCs), human parthenogenetic stem cells (hpSCs), dental pulp stem cells (DPSCs) etc.), if the grafts should be autologous or allogeneic, the best target area, as well as the identification of the key parameters that determine if the graft will lead to functional recovery (Barbuti et al., 2021; Xiao et al., 2021).

From the first attempts, up to the vast majority of current studies in PD, grafts are intrastriatal. The aim is to replace dopamine in the target brain area, without reconstructing the nigrostriatal pathway. Although some of these studies led to significant improvement of motor function, intrastriatal grafts have many limitations. Because the nigrostriatal pathway is not reconstructed, even if the motor symptoms of the disease are alleviated, the non-motor symptoms, that severely affect the patient's quality of life, are not significantly improved. Moreover, intrastriatal grafts lack physiological stimulus regulation, exhibiting tonic dopamine release, that is not sufficient to maintain improvement. Lastly, the clinical trials using fetal ventral midbrain intrastriatal grafts, that were held in the 2000s revealed significant graft-induced side effects (e.g. dyskinesias), possibly due to the contamination of the graft-

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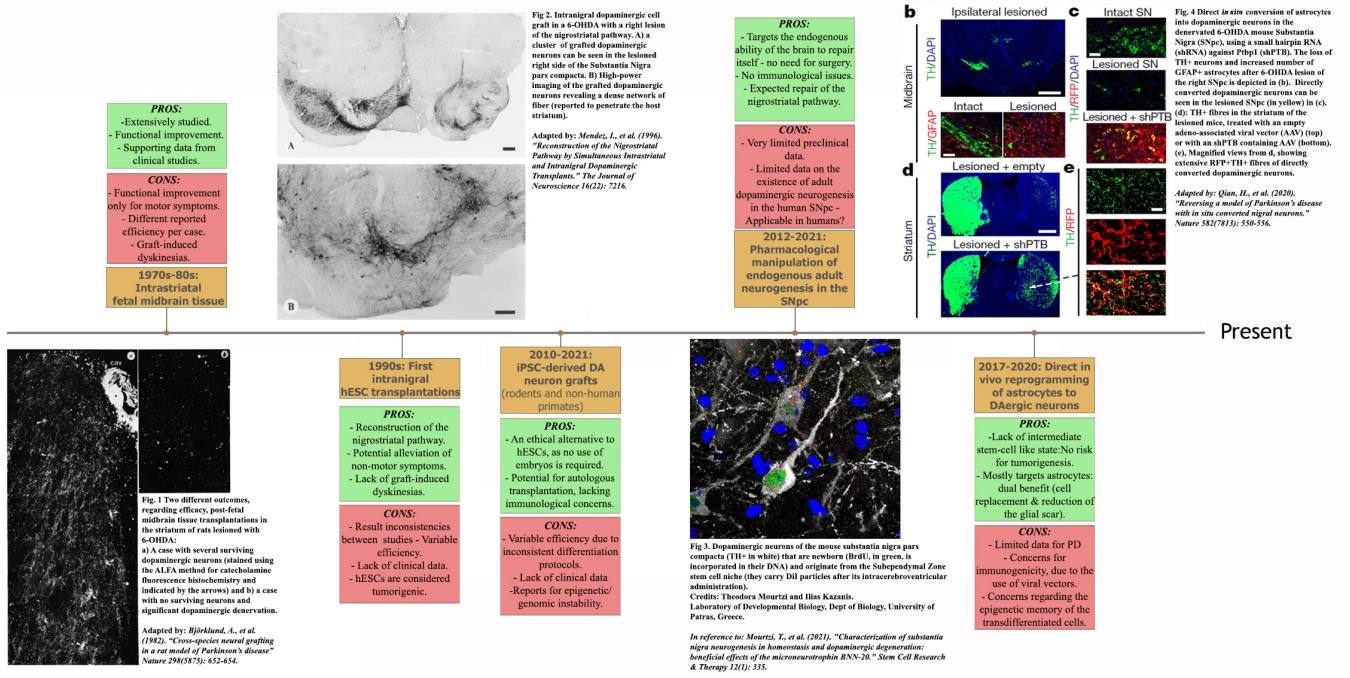

Figure 1 | **Timeline of the major milestones of cell replacement therapies used for the treatment of Parkinson's disease.**
The achievements (in green) and failures (in red) of each clinical/experimental approach are described in brief. DAPI: 4′,6-Diamidino-2-phenylindole; GFAP: glial fibrillary acidic protein; hESCs: human embryonic stem cells; PD: Parkinson's disease; shPTB: small hairpin polypyrimidine tract binding protein; SN: substantia nigra; SNpc: substantia nigra pars compacta; TH^+: tyrosine hydroxylase-positive.

derived neurons with non-dopaminergic neuronal types (e.g. serotoninergic) (Björklund and Parmar, 2021).

On that basis, intranigral transplantations are more appealing, as they might allow the restoration of the nigrostriatal pathway and, consequently, of the full basal ganglia circuitry. The first experimental studies using solely intranigral grafts did not have good results, as transplanted cells failed to recreate the nigrostriatal pathway (Mendez et al., 1996; Mukhida et al., 2001). In these studies, significant improvement in the restoration of the nigrostriatal tract was observed after "bridging" the substantia nigra and the striatum with grafts (Dunnett et al., 1989) or chemical substances like ibotenic or kainic acid (Zhou and Chiang, 1995), or by simultaneous intrastriatal and intranigral cell transplantations (Mendez et al., 1996; Baker et al., 2000; Mukhida et al., 2001). These preclinical findings led to a pilot clinical study, by the Mendez research group, that included three idiopathic PD patients and which generated with promising results (Mendez et al., 2002). Nevertheless, this strategy has not been followed up by additional clinical trials. During the last decade, a number of experimental studies revisited the intranigral transplantation approach with remarkable results. hESC intranigral grafts in the 6-OHDA PD mouse model showed good results in terms of graft survival, circuitry repair and of physiological regulation of dopamine release. Targeting the midbrain (SNpc and ventral tegmental area) also presents two additional advantages compared to the striatum: Firstly, the presence of other types of graft-derived neurons (such as GABAergic and glutamatergic neurons) does not cause side effects because they are normally present in these areas and appear to project towards their physiological target areas in the forebrain, completely avoiding the striatum (Björklund and Parmar, 2021). Secondly, recent studies have indicated that the SNpc is more permissive for neurogenesis, compared to the striatum; thus, its microenvironment could foster increased rates of neuronal differentiation and survival of the grafts (Maya-Espinosa et al., 2015; Collazo-Navarrete et al., 2019).

However, it should be noted that while in rodents intranigral graft-derived dopaminergic neurons can project successfully to the striatum (that is located approximately 8–10 mm rostrally), in humans and non-human primates the distance between the SNpc and the striatum is significantly greater (about 10-fold); therefore the ability of new nigral processes to reach the striatum might be impeded (Fan et al., 2020; Björklund and Parmar, 2021).

Although recent experimental work has strengthened significantly the prospect of overcoming the limitations that hindered the success of therapeutic strategies tested during the early "era of transplantations" for treating PD, there are still numerous issues to be solved. First of all, a systematic determination of the optimal graft type is lacking. Most of the recent intranigral graft studies involved hESCs which, albeit appearing to be very effective, hold a great risk for tumorigenesis. Undifferentiated hESCs are reported to induce teratomas or even malignant teratocarcinomas (Blum and Benvenisty, 2008; Doi et al., 2012); for example post-hESC grafting in a primate model of PD (Doi et al., 2012). A common strategy to constrain this problem is to pre-differentiate hESCs towards dopaminergic neurons (Doi et al., 2012; Guo et al., 2021), and/or to devise strategies to remove potentially oncogenic cells prior or after the transplantation, for example by the use of compounds that are specifically toxic to undifferentiated hESCs (Schriebl et al., 2011; Lin et al., 2017), or by using cell sorting protocols (Schriebl et al., 2011).

Recently, parthenogenetic hESCs (hpSCs) -that are generated after oocyte activation without sperm fertilization- have gained attention as a more ethical source of hESCs, the use of human embryos is not necessary (Volarevic et al., 2018; Wang et al., 2018). hpESC cell lines of the appropriate clinical grade have been assessed in primate and non-primate preclinical PD models, leading in two ongoing clinical trials for the evaluation of safety and efficacy (NCT02452723 and NCT03119636) (Garitaonandia et al., 2016; Wang et al., 2018). The first study is a Phase I trial, aiming to investigate the safety, tolerability and biodistribution of hpSC-derived neural stem cell (NSC) transplantations in the striatum and the substantia nigra of PD patients (Garitaonandia et al., 2016), while the second is a Phase I/II trial aiming in evaluating the efficacy of hpSC-derived committed dopaminergic neural cell transplantations in the striatum (Wang et al., 2018). Regarding the work that led to the second trial, preclinical investigations in monkeys provided proof of safety and tolerability and although the viability of the grafts was low, it led to a slight increase of dopamine in the striatum, accompanied by strong locomotor improvement, especially when the grafts were placed with precision in both the caudate nucleus and the putamen. However, the authors reported big variability between the animals in terms of behavioral measures of recovery (Wang et al., 2018).

The use of human induced pluripotent stem cells (hiPSCs) has gained attention as a promising and ethical alternative to hESCs and significant progress has been made in optimizing the protocols applied for the generation of hiPSCs, in order to reduce the risk of insertional mutagenesis, tumorigenesis and teratogenesis. Hargus et al. (2010) performed intrastriatal grafts in 6-OHDA mice, using PD patient iPSC-derived dopaminergic neurons and reported good viability, arborization and behavioral improvement. A later study by Sundberg et al. (2013), showed similar results in 6-OHDA rats using non-human primate iPSC-derived dopaminergic neurons. Those observations were also replicated by autologous grafts in one 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated macaque (Sundberg et al., 2013). Later on, a number of studies in PD non-human primate models have shown that autologous, but not allogeneic intrastriatal grafts of iPSC-derived dopaminergic neurons/progenitors can promote functional recovery (Hallett et al., 2015; Wang et al., 2015; Tao et al., 2021). More studies are needed to assess issues of efficiency and stability in iPSC-based cell therapies, caused by the use of distinct iPSC differentiation protocols (Zeng and Couture, 2013), as well as to epigenetic modifications. For example, it has been proposed that human (in contrast to mouse-derived) iPSCs retain X-chromosome inactivation during early passages but, later, they exhibit variable patterns of X-chromosome reactivation that can lead to genomic instability (Andoh-Noda et al., 2017; Sonntag et al., 2018). Moreover, the extent of epigenetic memory in iPSCs remains controversial, but can impact significantly on their pluripotency and their capacity for efficient differentiation, especially towards cell lineages different from the host tissue (Sonntag et al., 2018; Chen et al., 2019a). Issues of genomic resulted in the cancellation of the first clinical trial using iPSCs in humans, which started in 2014 for the

treatment age-related macular degeneration (Garber, 2015; Sonntag et al., 2018). The study was cancelled because three copy-number variations (CNVs) and three single nucleotide variations (SNVs) were identified in the first patient that received the experimental therapy, even though no related side effects were observed (Garber, 2015; Sonntag et al., 2018). This instability has been attributed to several factors/mechanisms (extensively reviewed elsewhere) such as: pre-existing somatic mutations of the host tissue cells, the differentiation protocols (especially when viral vectors are used to induce pluripotency) or mutations resulting from the reprogramming process itself (Yoshihara et al., 2017; Sonntag et al., 2018). Postnatal NSCs, on the other hand, have shown very promising efficacy and safety results in experimental animals, but are impossible to harvest in humans (Chen et al., 2019b).

Many efforts have been made to improve the outcome of transplantation strategies. It has been proposed that the hostile microenvironment of the degenerated tissue, which is present in both human PD and toxic models (Trist et al., 2019), might be a detrimental contributor in cases of graft inefficacy (Sakata et al., 2012b; Othman and Tan, 2020). The host microenvironment exerts neuroinflammatory and oxidative stress on the graft that can impair the survival, differentiation and maturation/integration of NSCs and NSC-derived immature neurons (Sakata et al., 2012b; Othman and Tan, 2020). Oxidative stress, in particular, can exert a dual role in NSCs. Low doses of reactive oxygen species (ROS) are part of the normal proliferation/differentiation mechanisms, promoting epigenetic regulation and modulating cell fate decision of NSCs (Tay et al., 2021), including the dopaminergic phenotype (Dreyer-Andersen et al., 2018). On the other hand, high levels of ROS have a negative impact on transplanted NSCs, reducing their neuroreparative efficacy (Kahroba et al., 2021). This is why, several studies in central nervous system (CNS) injury models have demonstrated that pre-treatment of NSCs with antioxidant factors can increase the viability of NSC grafts and the overall success of the intervention. Although limited data are available in PD models, such as on the use of melatonin to increase transplantation efficacy (Sharma et al., 2007; Mendivil-Perez et al., 2017), many antioxidant compounds have been used in NSC transplantations in experimental models of stroke. Some examples of molecules that have been tested are antioxidant enzymes (Sakata et al., 2012a; Wakai et al., 2014), melatonin (Mendivil-Perez et al., 2017), the Nrf2-inducing compounds minocycline and doxycycline (Kahroba et al., 2021), advanced nanomaterials (Jiang et al., 2019; Yu et al., 2020), as well as the neurotrophic factor brain-derived neurotrophic factor (BDNF) (Ma et al., 2012; Rosenblum et al., 2015). This suggests that relevant research is necessary in order to assess if pretreatment with antioxidants could also increase the success rate of CRTs in PD, as well.

It should be noted that most of the studies investigating the effect of intrastriatal grafts and all the intranigral graft studies, have been performed in chemotoxic models of PD (mainly the 6-OHDA and MPTP models). This is probably due to the lack of significant dopaminergic cell loss in most of the available genetic models. The only exception, is a small number of studies in which intrastriatal grafts of various ESC-derived cell types (embryoid bodies, neural stem cells, neural progenitors, differentiated neurons) were performed in the Pitx3 deficient "aphakia" mouse model of PD, showing significant motor and cognitive improvement (Chung et al., 2011, 2014; Moon et al., 2013; Zenchak et al., 2020). Therefore, cell replacement experimental work relies heavily on chemotoxic models of PD, with the significant caveat of the existence of extremely high levels of acute oxidative stress, that can negatively impact the outcome of the transplantation.

Another strategy to improve the outcome of cell replacement is the addition of neurotrophic support to the graft, prior and/or after the transplantation, which can increase its success rate. Neurotrophic factors such as BDNF, glialcellline-derived neurotrophic factor (GDNF) and nerve growth factor can promote the survival, differentiation, and migration of grafted cells and increase the success rate, for example by enhancing the functionality of the graft, both in animal models (Marsh and Blurton-Jones, 2017) and in humans. During one of the first transplantation clinical trials, grafts that where pre-treated with GDNF exhibited significantly improved functionality (107% increase in putaminal fluorodopa uptake; Mendez et al., 2000).

As neurotrophins appear to be beneficial for the treatment of PD *per se*, the combination of transplantation and neurotrophin administration could represent a promising multitargeting PD therapy. During the last years the effort to find new micromolecular mimetics of endogenous neurotrophins, that can penetrate the blood-brain barrier in order to allow systemic administration has gained pace (Gravanis et al., 2017). In a series of recent reports, we have shown that the microneurotrophin BNN-20, a synthetic analogue of DHEA, mimics BDNF through the selective activation of its receptor TrkB and exhibits strong neuroprotective (Botsakis et al., 2017; Panagiotakopoulou et al., 2020) and neurogenic (Mourtzi et al., 2021) effects in the SNpc of the "weaver" mouse model of PD. It will be interesting to assess the potentially beneficial effects of neurotrophins in the survival and repair capacity of grafted cells.

Direct *In Vivo* Reprogramming

The iPSC technology opened new prospects for a graft-based CRT in PD, as it provided a new source of autologous cells. However, in depth characterization and preclinical testing of iPSCs revealed numerous drawbacks. Firstly, as the generation of iPSC requires the de-programming of cells towards a pluripotent stem cell state, iPSCs exhibit increased risk for tumorigenesis and teratogenesis. Moreover, the successful differentiation of iPSCs towards the desired cell fate is a multi-step process that requires precise and time-specific manipulation of gene expression, making it difficult to achieve at a reproducible, controlled and clinically-relevant level (Chen et al., 2019b). Transdifferentiation or direct reprogramming constitutes a very attractive alternative, as it does not require the transition through an intermediate stem-cell like state (Bocchi and Götz, 2020) and, lately, is often performed at a single step (Qin et al., 2020; Zhou et al., 2020).

In neurodegenerative diseases or CNS injury, *in vivo* direct reprogramming serves two key purposes: cell replacement (typically of neurons) and the attenuation of adverse immunological effects and of the formation of the glial scar, that is typically formed in areas of lesion. The latter is because transdifferentiation targets astrocytes, an abundant CNS cell population that is mostly responsible for the aforementioned complications (Bocchi and Götz, 2020).

In 2011, Pfisterer et al. reported the direct reprogramming of human embryonic and postnatal fibroblasts into dopaminergic neurons *in vitro*, by the overexpression of Ascl1, Brn2 and Mytl1, complemented by Lmx1a and FoxA2 (two crucial pro-dopaminergic transcription factors) (Pfisterer et al., 2011). The first successful *in vivo* dopaminergic transdifferentiation came in 2017, when Rivetti di Val Cervo et al., directly reprogrammed *in vivo* adult striatal mouse astrocytes into functional dopaminergic neurons, in the 6-OHDA PD mouse model by overexpressing three transcription factors, NeuroD1, Ascl1 and Lmx1a, and the microRNA miR218 (Rivetti di Val Cervo et al., 2017). Three months later, Yoo et al. also reported direct conversion of adult striatal astrocytes into dopaminergic neurons in the MPTP mouse model using a different approach. They combined electromagnetic field stimulation and gold nanoparticles to activate the histone acetyltransferase Brd2, leading to histone H3K27 acetylation and to the activation of neuron-specific genes (Yoo et al., 2017). However, as in these two cases induced dopaminergic neurons were generated in the striatum and not in the SNpc, it is expected that the same limitations as intrastriatal stem cell grafts (graft-induced dyskinesias and lack of dopamine release regulation) would be anticipated.

A recently published paper by Qian et al. (2020) reported impressive evidence for *in vivo* transdifferentiation of astrocytes in the midbrain of the 6-OHDA mouse model into functional dopaminergic neurons, leading to the repair of the nigrostriatal circuit, the improvement of dopamine release in the striatum and to almost full alleviation of motor symptoms. Their in depth analysis confirmed that the newly-generated dopaminergic neurons projected to the striatum (Qian et al., 2020).

Reprogramming was achieved by inserting a short-hairpin RNA against the RNA polypyrimidine tract binding protein (PTB)-1 using an adeno-associated viral vector in 2-month-old 6-OHDA mice. Although the restoration of the dopaminergic projection was only partial (30% of control), it led to the restoration of dopamine in the striatum to 65% of control levels and to almost full alleviation of motor symptoms (using apomorphine and amphetamine-induced rotation and limb-use asymmetry testing). Notably, when this protocol was applied in 1 year old mice (to resemble the age of late onset PD in humans) it led to a significant improvement in limb-use asymmetry, but failed to improve apomorphine-induced rotation (Qian et al., 2020). Although the authors do not mention if the inefficacy seen in older animals is due to a reduced astrocyte-neuron conversion rate, or due to the limited functionality/arborization of the induced neurons, this finding suggests that age-dependent differences could affect the outcome of a cell therapeutic strategy especially as sporadic PD (accounting for almost 85% of PD cases) appears in older individuals (Tran et al., 2020).

The data on the molecular events that interfere with successful astrocyte to neuron conversion in aging animals are limited, but many mechanisms have been implicated in direct fibroblast to neuron conversion insufficiency in older human cells (Böhnke et al., 2018). For example, the epigenetic age, as seen by the DNA methylation pattern is maintained during transdifferentiation. Moreover, as direct conversion does not include cell division, the macromolecules of the parental cells are not diluted, hence, their damage that accumulates with age can interfere with successful conversion (Böhnke et al., 2018). In combination with the reported mitochondrial dysfunction (reduced gene transcription and decreased oxidative phosphorylation) seen in older fibroblasts and induced neurons, it is obvious that the increased ROS content leads to damage that is retained in older induced neurons. The most pronounced of these changes are the increased DNA damage (as seen by longer comet tails in older induced neurons) and the nuclear pore insufficiency. Nucleoporins, the structural component of nuclear pores become leaky with age and importin β family proteins, such as RANPB17 are downregulated, resulting in reduced nucleo-cytoplasmic compartmentalization. We can hypothesize that similar changes can also occur during astrocyte-neuron direct conversion in older mice, impeding the success of the intervention (Mertens et al., 2015; Böhnke et al., 2018).

PTB downregulation, using a CRISPR-CasRx approach, has been also used to directly convert striatal astrocytes to dopaminergic neurons in the 6-OHDA model, again with high efficiency of conversion and accompanied by significant motor improvement (Zhou et al., 2020).

Taken together, these recent results provide high hope for a future *in vivo* transdifferentiation therapy in PD, also strengthened by the fact that it is not suspected for tumorigenesis. Direct reprogramming was also expected to be void of immune-related rejection complications, since it involves endogenous cells. However, adverse immune responses have been observed in some

cases, attributed to the viral vectors used for the manipulation of gene expression.

Although adeno-associated viral vector [as the ones used in the papers of Qian et al. (2020) and Zhou et al. (2020)] appear to be less immunogenic that lentiviral and retroviral vectors, they are less specific for glial cells and can also enter neurons, a fact that could affect the interpretation of results. Thus, additional confirmation that glial cells are, in fact, converted to neurons is needed (Bocchi and Götz, 2020). Qian et al. (2020) used a chemogenic DREADD approach and clearly showed that activity-induced signaling by astrocyte-derived neurons was responsible for the observed phenotypic recovery.

Another important issue regarding transdifferentiation is the epigenetic signature. During iPSCs induction most epigenetic modifications are reversed to an embryonic-like state. However, the unreversed epigenetic landscape (consisting of what is called "epigenetic memory") could affect the phenotype and function of iPSCs and of iPSC-derived neurons. This issue is much more complex in transdifferentiation protocols, in which more specific epigenetic signatures are modified in order to achieve cell-type conversion; thus, the epigenetic memory is retained to a greater extent. This means that induced neurons retain a large part of the epigenetic memory of their ancestor cell, including environmental and age-dependent modifications (Stricker and Götz, 2021). Although this fact could be beneficial when direct reprogramming is used for experimental disease modelling (as it can better retain the environmental and age-related elements of the pathological phenotype) (Samoylova and Baklaushev, 2020), it consists an important issue for therapy, possibly appearing only in specific conditions. It will be necessary to harvest the reprogrammed cells in order to perform single-cell epigenetic memory assessments (Stricker and Götz, 2021).

Finally, regarding the scale-up from rodents to humans, the same issue mentioned in intranigral transplantations, also applies here. The length of the nigrostriatal tract in humans is about 10-fold the one in rats, so, cell replacement strategies that appear effective in rodents may be insufficient or require additional methods (e.g. neurotrophic support) in humans (Björklund and Parmar, 2021).

Manipulation of Substantia Nigra Neurogenesis

An almost ideal CRT strategy in neurodegenerative diseases (including PD) would be to manipulate endogenous mechanisms of the adult brain to repair itself (Fan et al., 2020).

After two major neurogenic niches were identified in the postnatal mammalian brain [the subgranular zone of the dentate gyrus of the hippocampus and the subependymal layer lining the lateral wall of the lateral ventricles (SEZ)] the prospect of enhancing the endogenous regenerative capacity of the brain became realistic (Shohayeb et al., 2018). Many non-typical neurogenic areas, such as in the hypothalamus, the striatum, the substantia nigra and the cortex, have been also reported (Feliciano et al., 2015; Jurkowski et al., 2020), providing additional ground for the development of such therapeutic approaches. These areas, often referred to as "non classical" or "non-canonical" neurogenic areas, exhibit neurogenic activity in normal conditions, after ischemic injury or under degenerative conditions, and, although they are expected to provide area-specific neurogenesis, their cell-differentiation potential is still under characterization (Jurkowski et al., 2020).

Specifically for the Substantia Nigra, while the existence of adult-born cells has been almost universally accepted, there were significant inconsistencies regarding the phenotypic identity of those cells. Few research teams reported dopaminergic neurogenesis in wild-type conditions (Zhao et al., 2003; Van Kampen and Robertson, 2005; Shan et al., 2006; Xie et al., 2017) and in the degenerated SNpc of PD rodent models (Zhao et al., 2003; Shan et al., 2006; Xie et al., 2017), while others reported differentiation only towards the glial identity (Mao et al., 2001; Lie et al., 2002; Frielingsdorf et al., 2004; Padel et al., 2016). The existence of local progenitors capable of differentiating towards neurons *in vitro*, or when grafted in the neurogenic niche of the hippocampus had been also reported (Lie et al., 2002), with a separate group of studies suggesting that a small number of dopaminergic neurons can be differentiated from a group of nestin$^+$/sox2$^-$ cells residing in the adult midbrain (Albright et al., 2016; Farzanehfar et al., 2017; Xie et al., 2017). While these findings are not confirmed for the human brain, an older study by Yoshimi et al. (2005) revealed the existence of PSA$^+$ cells in the SNpc and the SN pars reticulata (SNr) of post-mortem human brains of healthy individuals, as well as PD patients, which could imply the generation of new neurons in the area. Moreover, the generation of new neurons in non-classical areas of the adult to demonstrate the birth of new striatal neurons in humans (Ernst et al., 2014). This means that while adult neurogenesis in the human SNpc is not proven, the possibility that it exists remains valid, and worthy of future evaluation.

The generation of newborn dopaminergic neurons in the adult SNpc of both healthy and "weaver" mice has been demonstrated using BrdU cell-tracing protocols (Mourtzi et al., 2021). Interestingly, these newborn dopaminergic neurons appear to retain a higher level of resilience to degeneration, compared to "older" neurons and their maturation follows the canonical mesencephalic differentiation pathway, as judged by the expression of FoxA2 (a key midbrain transcription factor). The exogenous manipulation of this system by the administration of the microneurotrophin BNN-20 enhanced neurogenesis specifically in the SNpc (Mourtzi et al., 2021) and led to a three-fold increase of dopamine in the striatum, and to significant locomotor improvement (Botsakis et al., 2017; Panagiotakopoulou et al., 2020; Mourtzi et al., 2021).

A similar strategy had been proposed earlier by Adeosun et al. (2012), based on the neurosteroid small-molecule allopregnanolone. Exogenous allopregnanolone administration led to the significant increase in the generation of new dopaminergic neurons in the SNpc of the MPTP mouse model, and to the subsequent restoration of the MPTP-induced motor deficits. However, it did not reverse the loss of dopamine in the striatum, with the authors attributing the functional recovery to the allopregnanolone-induced increase of norepinephrine in the striatum (Adeosun et al., 2012). Similar, promising, observations have been documented in transgenic models of Alzheimer's disease, that also exhibit a reduction of the dopaminergic cell number in the SNpc, prior to the appearance of b-amyloid (Ab)-positive plaques (Sun et al., 2012; Zhang et al., 2015): the triple transgenic (3xTgAD) and the APPswe/PSEN1 double transgenic mouse (2xTgAD) models. In these studies, the exogenous administration of allopregnanolone resulted in an increased rate of adult dopaminergic neurogenesis in the SNpc. Based on the above, it will be very interesting to test more extensively the pro-neurogenic effects of allopregnanolone in additional PD models.

Although the available data is still sparse, the development of small-molecule drugs aiming to manipulate endogenous neurogenesis constitutes an attractive neuroregenerative target (Herrera-Arozamena et al., 2016; Mourtzi et al., 2021). Nevertheless, the absolute prerequisite for such cell replacement strategies is the existence of endogenous neurogenesis. Our and other's experimental work suggests that dopaminergic neurogenesis in the SN relies, at least partially, to the SEZ (Zhao et al., 2003; Xie et al., 2017; Mourtzi et al., 2021). A number of recent studies have shown that the ability of the human SEZ to give rise to neuroblasts declines rapidly after the 18th month of age (Goritz and Frisen, 2012; Bergmann et al., 2015). On the other hand, earlier work has showed that the remaining neuroblasts of the adult human SEZ retain active proliferation (Wang et al., 2011) and can migrate to remote areas of lesion, for example in vascular dementia (Ekonomou et al., 2011). Moreover, the presence of immature neurons (including tyrosine hydroxylase-positive) has been reported in the SN of PD patients (Yoshimi et al., 2005) and neural progenitor cells able for *in vitro* proliferation and neuronal differentiation have been isolated from the SN of PD patients, postmortem (Wang et al., 2012), while the activity of the SEZ has be reported to be maintained in human PD patients to the levels of age-matched healthy individuals (van den Berge et al., 2011). Importantly, few studies investigating the injured cortex and SN have reported that latent parenchymal progenitors can become activated in the context of a degenerative microenvironment, exhibiting a prominently neurogenic output (Lie et al., 2002; Sirko et al., 2013; Mourtzi et al., 2021).

What Determines the Functionality of the Newly Generated Neurons?

The ultimate goal of a cell replacement strategy against PD is to alleviate both the motor and the non-motor symptoms. This relies on achieving good functionality of different types of newly generated neurons, which requires high rates of survival and of successful integration into the existing neuronal circuits.

In the case of cell grafts, only a small fraction of cells survives and differentiates into dopaminergic neurons. The determination of the minimum number of new dopaminergic neurons that are necessary to achieve significant locomotor improvement is difficult, especially in intrastriatal grafts (Fan et al., 2020). It has been estimated that even the addition of only 650 new dopaminergic neurons in the rat striatum (Rath et al., 2013) and of 16,000 new dopaminergic neurons in the macaque brain is sufficient for motor improvement (Kikuchi et al., 2011, 2017); while, 2–4 million grafted cells are needed to produce a range of 16,000–100,000 viable dopaminergic neurons in the non-human rimates (Barbuti et al., 2021).

On the other hand, in the case of intranigral grafts or in transdifferentiation approaches, it has been easier to quantify the minimum amount of new dopaminergic neurons that are required to achieve motor improvement, as both the number of SNpc dopaminergic cells and of their projections to the striatum can be quantified and correlated to normal conditions.

Interestingly, only a partial restoration of the SNpc dopaminergic cell number can produce significant, or even full, motor function. In the 6-OHDA-lesioned SNpc, the partial restoration of the dopaminergic cell number, using the direct *in situ* conversion of nigral astrocytes to dopaminergic neurons, only to 33% of the control, resulted in a relatively larger increase in the levels of dopamine in the striatum (to 65% of control) and to an almost full functional restoration of rotational behavior and limb-use asymmetry tests (Qian et al., 2020). This observation could imply an upregulation in dopamine synthesis in the newly generated dopaminergic neurons and suggests that even a partial restoration of the dopaminergic cell number in the SNpc could lead to significant functional improvement. Similarly, in a study of intranigral cell transplantations in the 6-OHDA mouse the restoration of the SNpc dopaminergic cell number at the 40% of the intact tissue, led to the significant increase of dopamine levels in the striatum (albeit remaining 77% below control levels), followed by the significant reduction of rotational defects by 94%, two months after the transplant (Gaillard et al., 2009). In agreement

to the above, we have previously reported that long-term administration of BNN-20 led to a partial restoration of the dopaminergic cell number in the SNpc to approximately 63% of control and resulted in an almost 3-fold increase of dopamine levels in the striatum (albeit, still remaining 74% below control levels), followed by a significant amelioration in motor behavior using the open-field assessment (Panagiotakopoulou et al., 2020).

Finally, it should be cautioned that in the case of intranigral grafts or nigral *in situ* transdifferentiation, it is very important to allow enough time post-intervention to allow for the maximum number of projections towards the striatum. Two studies that incorporated various time points after the cell replacement intervention reported that a time frame of 3 months, but not 1 month, post *in situ* transdifferentiation (Qian et al., 2020) and 16 weeks, but not 6 weeks, post intranigral transplantation (Thompson et al., 2009) were necessary to achieve the maximum restoration of the striatal dopaminergic innervation. During the last decade, several efforts have been made to utilize biomaterials (e.g. polymeric or gelatin scaffolds), in order to increase the survival and differentiation of the transplanted stem cells/progenitors, or to enhance the axonal outgrowth, arborization and engraftment of the newly generated neurons to promote tissue (Du et al., 2015; Mitrousis et al., 2018; Shin et al., 2018). Such applications could play a pivotal role in the development of a successful cell replacement strategy in the future.

Conclusions

Even though the various cell transplantation therapies against PD have a lot of issues to resolve before a successful and safe clinical application, a lot of progress has been made (and continues to evolve) in the last decade.

The cell replacement approaches that aim to restore the physiological nigrostriatal circuitry appear to be the most promising of all. Hence, intranigral cell transplantations and, even more, direct reprogramming of nigral non-neuronal cells into dopaminergic neurons are more attractive due to their increased efficacy and reduced side-effects compared to the "traditional" intrastriatal cell grafts. However, as these approaches are relatively new, we are still quite far from a viable clinical approach.

It is worth to mention that a cell replacement approach that would target the purely endogenous neurogenic potential of the adult substantia nigra would be ideal for the treatment of PD, as it could resolve many of the safety issues of the other CRT strategies. Hence, it is very important to evaluate its proof of concept for the human PD in the future, as it could represent a valuable alternative.

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References

Adeosun SO, Hou X, Jiao Y, Zheng B, Henry S, Hill R, He Z, Pani A, Kyle P, Ou X, Mosley T, Farley JM, Stockmeier C, Paul I, Bigler S, Brinton RD, Smeyne R, Wang JM (2012) Allopregnanolone reinstates tyrosine hydroxylase immunoreactive neurons and motor performance in an MPTP-lesioned mouse model of Parkinson's disease. PLoS One 7:e50040.
Albright JE, Stojkovska I, Rahman AA, Brown CJ, Morrison BE (2016) Nestin-positive/SOX2-negative cells mediate adult neurogenesis of nigral dopaminergic neurons in mice. Neurosci Lett 615:50-54.
Andoh-Noda T, Akamatsu W, Miyake K, Kobayashi T, Ohyama M, Kurosawa H, Kubota T, Okano H (2017) Differential X chromosome inactivation patterns during the propagation of human Induced pluripotent stem cells. Keio J Med 66:1-8.
Baker KA, Sadi D, Hong M, Mendez I (2000) Simultaneous intrastriatal and intranigral dopaminergic grafts in the parkinsonian rat model: Role of the intranigral graft. J Comp Neurol 426:106-116.
Barbuti PA, Barker RA, Brundin P, Przedborski S, Papa SM, Kalia LV, Mochizuki H (2021) Recent advances in the development of stem-cell-derived dopaminergic neuronal transplant therapies for Parkinson's disease. Mov Disord 36:1772-1780.
Bergmann O, Spalding KL, Frisén J (2015) Adult neurogenesis in humans. Cold Spring Harb Perspect Biol 7:a018994.
Björklund A, Stenevi U, Dunnett SB, Iversen SD (1981) Functional reactivation of the deafferented neostriatum by nigral transplants. Nature 289:497-499.
Björklund A, Stenevi U, Dunnett SB, Gage FH (1982) Cross-species neural grafting in a rat model of Parkinson's disease. Nature 298:652-654.
Björklund A, Parmar M (2021) Dopamine cell therapy: from cell replacement to circuitry repair. J Parkinsons Dis 11:S159-165.
Blum B, Benvenisty N (2008) The tumorigenicity of human embryonic stem cells. Adv Cancer Res 100:133-158.
Bocchi R, Götz M (2020) Neuronal reprogramming for brain repair: challenges and perspectives. Trends Mol Med 26:890-892.
Böhnke L, Traxler L, Herdy JR, Mertens J (2018) Human neurons to model aging: A dish best served old. Drug Discov Today Dis Models 27:43-49.
Botsakis K, Mourtzi T, Panagiotakopoulou V, Vreka M, Stathopoulos GT, Pediaditakis I, Charalampopoulos I, Gravanis A, Delis F, Antoniou K, Zisimopoulos D, Georgiou CD, Panagopoulos NT, Matsokis N, Angelatou F (2017) BNN-20, a synthetic microneurotrophin, strongly protects dopaminergic neurons in the "weaver" mouse, a genetic model of dopamine-denervation, acting through the TrkB neurotrophin receptor. Neuropharmacology 121:140-157.
Brundin P, Nilsson OG, Gage FH, Björklund A (1985) Cyclosporin A increases survival of cross-species intrastriatal grafts of embryonic dopamine-containing neurons. Exp Brain Res 60:204-208.
Chen S, Wang JM, Irwin RW, Yao J, Liu L, Brinton RD (2011) Allopregnanolone promotes regeneration and reduces β-amyloid burden in a preclinical model of Alzheimer's disease. PLoS One 6:e24293.
Chen S, Zhang J, Zhang D, Jiao J (2019a) Acquisition of functional neurons by direct conversion: switching the developmental clock directly. J Genet Genomics 46:459-465.
Chen S, Wang T, Yao J, Brinton RD (2020) Allopregnanolone promotes neuronal and oligodendrocyte differentiation in vitro and in vivo: therapeutic implication for Alzheimer's disease. Neurotherapeutics 17:1813-1824.
Chen W, Huang Q, Ma S, Li M (2019b) Progress in dopaminergic cell replacement and regenerative strategies for Parkinson's disease. ACS Chem Neurosci 10:839-851.
Chung S, Moon JI, Leung A, Aldrich D, Lukianov S, Kitayama Y, Park S, Li Y, Bolshakov VY, Lamonerie T, Kim KS (2011) ES cell-derived renewable and functional midbrain dopaminergic progenitors. Proc Natl Acad Sci U S A 108:9703-9708.
Chung S, Moon J, Kim KS (2014) Improvement of neurological dysfunctions in aphakia mice, a model of Parkinson's disease, after transplantation of ES cell-derived dopaminergic neuronal precursors. Methods Mol Biol 1213:285-291.
Collazo-Navarrete O, Hernández-García D, Guerrero-Flores G, Drucker-Colín R, Guerra-Crespo M, Covarrubias L (2019) The substantia nigra is permissive and gains inductive signals when lesioned for dopaminergic differentiation of embryonic stem cells. Stem Cells Dev 28:1104-1115.
De Gioia R, Biella F, Citterio G, Rizzo F, Abati E, Nizzardo M, Bresolin N, Comi GP, Corti S (2020) Neural stem cell transplantation for neurodegenerative diseases. Int J Mol Sci 21:3103.
Doi D, Morizane A, Kikuchi T, Onoe H, Hayashi T, Kawasaki T, Motono M, Sasai Y, Saiki H, Gomi M, Yoshikawa T, Hayashi H, Shinoyama M, Refaat MM, Suemori H, Miyamoto S, Takahashi J (2012) Prolonged maturation culture favors a reduction in the tumorigenicity and the dopaminergic function of human ESC-derived neural cells in a primate model of Parkinson's disease. Stem Cells 30:935-945.
Dreyer-Andersen N, Almeida AS, Jensen P, Kamand M, Okarmus J, Rosenberg T, Friis SD, Martínez Serrano A, Blaabjerg M, Kristensen BW, Skrydstrup T, Gramsbergen JB, Vieira HLA, Meyer M (2018) Intermittent, low dose carbon monoxide exposure enhances survival and dopaminergic differentiation of human neural stem cells. PLoS One 13:e0191207.
Du BL, Zeng X, Ma YH, Lai BQ, Wang JM, Ling EA, Wu JL, Zeng YS (2015) Graft of the gelatin sponge scaffold containing genetically-modified neural stem cells promotes cell differentiation, axon regeneration, and functional recovery in rat with spinal cord transection. J Biomed Mater Res A 103:1533-1545.
Dunnett SB, Rogers DC, Richards SJ (1989) Nigrostriatal reconstruction after 6-OHDA lesions in rats: combination of dopamine-rich nigral grafts and nigrostriatal "bridge" grafts. Exp Brain Res 75:523-535.
Ekonomou A, Ballard CG, Pathmanaban ON, Perry RH, Perry EK, Kalaria RN, Minger SL (2011) Increased neural progenitors in vascular dementia. Neurobiol Aging 32:2152-2161.
Ernst A, Alkass K, Bernard S, Salehpour M, Perl S, Tisdale J, Possnert G, Druid H, Frisen J (2014) Neurogenesis in the striatum of the adult human brain. Cell 156:1072-1083.
Fan Y, Winanto, Ng SY (2020) Replacing what's lost: a new era of stem cell therapy for Parkinson's disease. Transl Neurodegener 9:2.
Farzanehfar P, Lu SS, Dey A, Musiienko D, Baagil H, Horne MK, Aumann TD (2017) Evidence of functional duplicity of Nestin expression in the adult mouse midbrain. Stem Cell Res 19:82-93.
Feliciano DM, Bordey A, Bonfanti L (2015) Noncanonical sites of adult neurogenesis in the mammalian brain. Cold Spring Harb Perspect Biol 7:a018846.
Frielingsdorf H, Schwarz K, Brundin P, Mohapel P (2004) No evidence for new dopaminergic neurons in the adult mammalian substantia nigra. Proc Natl Acad Sci U S A 101:10177.
Gaillard A, Decressac M, Frappé I, Fernagut PO, Prestoz L, Besnard S, Jaber M (2009) Anatomical and functional reconstruction of the nigrostriatal pathway by intranigral transplants. Neurobiol Dis 35:477-488.
Garber K (2015) RIKEN suspends first clinical trial involving induced pluripotent stem cells. Nat Biotechnol 33:890-891.
Garitaonandia I, Gonzalez R, Christiansen-Weber T, Abramihina T, Poustovoitov M, Noskov A, Sherman G, Semechkin A, Snyder E, Kern R (2016) Neural stem cell tumorigenicity and biodistribution assessment for phase I clinical trial in Parkinson's disease. Sci Rep 6:34478.
Goritz C, Frisen J (2012) Neural stem cells and neurogenesis in the adult. Cell Stem Cell 10:657-659.
Gravanis A, Pediaditakis I, Charalampopoulos I (2017) Synthetic microneurotrophins in therapeutics of neurodegeneration. Oncotarget 8:9005-9006.
Guo X, Tang L, Tang X (2021) Current developments in cell replacement therapy for Parkinson's disease. Neuroscience 463:370-382.
Hallett Penelope J, Deleidi M, Astradsson A, Smith Gaynor A, Cooper O, Osborn Teresia M, Sundberg M, Moore Michele A, Perez-Torres E, Brownell A-L, Schumacher James M, Spealman Roger D, Isacson O (2015) Successful function of autologous iPSC-derived dopamine neurons following transplantation in a non-human primate model of Parkinson's disease. Cell Stem Cell 16:269-274.
Hargus G, Cooper O, Deleidi M, Levy A, Lee K, Marlow E, Yow A, Soldner F, Hockemeyer D, Hallett PJ, Osborn T, Jaenisch R, Isacson O (2010) Differentiated Parkinson patient-derived induced pluripotent stem cells grow in the adult rodent brain and reduce motor asymmetry in Parkinsonian rats. Proc Natl Acad Sci U S A 107:15921-15926.
Herrera-Arozamena C, Martí-Marí O, Estrada M, De la Fuente Revenga M, Rodríguez-Franco MI (2016) Recent advances in neurogenic small molecules as innovative treatments for neurodegenerative diseases. Molecules 21:1165.
Jiang XC, Xiang JJ, Wu HH, Zhang TY, Zhang DP, Xu QH, Huang XL, Kong XL, Sun JH, Hu YL, Li K, Tabata Y, Shen YQ, Gao JQ (2019) Neural stem cells transfected with teactive oxygen species-responsive polyplexes for effective treatment of ischemic stroke. Adv Mater 31:1807591.
Jurkowski MP, Bettio L, K. Woo E, Patten A, Yau S-Y, Gil-Mohapel J (2020) Beyond the hippocampus and the SVZ: adult neurogenesis throughout the brain. Front Cell Neurosci 14:576444.
Kahroba H, Ramezani B, Maadi H, Sadeghi MR, Jaberie H, Ramezani F (2021) The role of Nrf2 in neural stem/progenitors cells: From maintaining stemness and self-renewal to promoting differentiation capability and facilitating therapeutic application in neurodegenerative disease. Ageing Res Rev 65:101211.
Kikuchi T, Morizane A, Doi D, Onoe H, Hayashi T, Kawasaki T, Saiki H, Miyamoto S, Takahashi J (2011) Survival of human induced pluripotent stem cell-derived midbrain dopaminergic neurons in the brain of a primate model of Parkinson's disease. J Parkinsons Dis 1:395-412.
Kikuchi T, Morizane A, Doi D, Magotani H, Onoe H, Hayashi T, Mizuma H, Takara S, Takahashi R, Inoue H, Morita S, Yamamoto M, Okita K, Nakagawa M, Parmar M, Takahashi J (2017) Human iPS cell-derived dopaminergic neurons function in a primate Parkinson's disease model. Nature 548:592-596.
Lie DC, Dziewczapolski G, Willhoite AR, Kaspar BK, Shults CW, Gage FH (2002) The adult substantia nigra contains progenitor cells with neurogenic potential. J Neurosci 22:6639.

Lin YT, Wang CK, Yang SC, Hsu SC, Lin H, Chang FP, Kuo TC, Shen CN, Chiang PM, Hsiao M, Lu FL, Lu J (2017) Elimination of undifferentiated human embryonic stem cells by cardiac glycosides. Sci Rep 7:5289.
Ma H, Yu B, Kong L, Zhang Y, Shi Y (2012) Neural stem cells over-expressing brain-derived neurotrophic factor (BDNF) stimulate synaptic protein expression and promote gunctional recovery following transplantation in rat model of traumatic brain injury. Neurochem Res 37:69-83.
Mao L, Lau YS, Petroske E, Wang JQ (2001) Profound astrogenesis in the striatum of adultmice following nigrostriatal dopaminergic lesion by repeated MPTP administration. Brain Res Dev Brain Res 131:57-65.
Marsh SE, Blurton-Jones M (2017) Neural stem cell therapy for neurodegenerative disorders: The role of neurotrophic support. Neurochem Int 106:94-100.
Maserejian N, Vinikoor-Imler L, Dilley A (2020) Estimation of the 2020 global population of Parkinson's disease (PD) [abstract]. Mov Disord 2020:35 (suppl 1).
Maya-Espinosa G, Collazo-Navarrete O, Millán-Aldaco D, Palomero-Rivero M, Guerrero-Flores G, Drucker-Colín R, Covarrubias L, Guerra-Crespo M (2015) Mouse embryonic stem cell-derived cells reveal niches that support neuronal differentiation in the adult rat brain. Stem Cells 33:491-502.
Mendez I, Sadi D, Hong M (1996) Reconstruction of the nigrostriatal pthway by simultaneous intrastriatal and intranigral dopaminergic transplants. J Neurosci 16:7216.
Mendez I, Dagher A, Hong M, Hebb A, Gaudet P, Law A, Weerasinghe S, King D, Desrosiers J, Darvesh S, Acorn T, Robertson H (2000) Enhancement of survival of stored dopaminergic cells and promotion of graft survival by exposure of human fetal nigral tissue to glial cell line--derived neurotrophic factor in patients with Parkinson's disease. Report of two cases and technical considerations. J Neurosurg 92:863-869.
Mendez I, Dagher A, Hong M, Gaudet P, Weerasinghe S, McAlister V, King D, Desrosiers J, Darvesh S, Acorn T, Robertson H (2002) Simultaneous intrastriatal and intranigral fetal dopaminergic grafts in patients with Parkinson disease: a pilot study: Report of three cases. J Neurosurg 96:589-596.
Mendivil-Perez M, Soto-Mercado V, Guerra-Librero A, Fernandez-Gil BI, Florido J, Shen YQ, Tejada MA, Capilla-Gonzalez V, Rusanova I, Garcia-Verdugo JM, Acuña-Castroviejo D, López LC, Velez-Pardo C, Jimenez-Del-Rio M, Ferrer JM, Escames G (2017) Melatonin enhances neural stem cell differentiation and engraftment by increasing mitochondrial function. J Pineal Res 63:e12415.
Mertens J, Paquola ACM, Ku M, Hatch E, Böhnke L, Ladjevardi S, McGrath S, Campbell B, Lee H, Herdy JR, Gonçalves JT, Toda T, Kim Y, Winkler J, Yao J, Hetzer MW, Gage FH (2015) Directly reprogrammed human neurons retain aging-associated transcriptomic signatures and reveal age-related nucleocytoplasmic defects. Cell Stem Cell 17:705-718.
Mitrousis N, Fokina A, Shoichet MS (2018) Biomaterials for cell transplantation. Nat Rev Mater 3:441-456.
Moon J, Lee HS, Kang JM, Park J, Leung A, Hong S, Chung S, Kim KS (2013) Stem cell grafting improves both motor and cognitive impairments in a genetic model of Parkinson's disease, the Aphakia (ak) mouse. Cell Transplant 22:1263-1279.
Mourtzi T, Dimitrakopoulos D, Kakogiannis D, Salodimitris C, Botsakis K, Meri DK, Anesti M, Dimopoulou A, Charalampopoulos I, Gravanis A, Matsokis N, Angelatou F, Kazanis I (2021) Characterization of substantia nigra neurogenesis in homeostasis and dopaminergic degeneration: beneficial effects of the microneurotrophin BNN-20. Stem Cell Res Ther 12:335.
Mukhida K, Baker KA, Sadi D, Mendez I (2001) Enhancement of sensorimotor behavioral recovery in hemiparkinsonian rats with intrastriatal, intranigral, and intrasubthalamic nucleus dopaminergic transplants. J Neurosci 21:3521.
Othman FA, Tan SC (2020) Preconditioning strategies to enhance neural stem cell-based therapy for ischemic stroke. Brain Sci 10:893.
Padel T, Özen I, Boix J, Barbariga M, Gaceb A, Roth M, Paul G (2016) Platelet-derived growth factor-BB has neurorestorative effects and modulates the pericyte response in a partial 6-hydroxydopamine lesion mouse model of Parkinson's disease. Neurobiol Dis 94:95-105.
Palasz E, Wysocka A, Gasiorowska A, Chalimoniuk M, Niewiadomski W, Niewiadomska G (2020) BDNF as a promising therapeutic agent in Parkinson's disease. Int J Mol Sci 21:1170.
Panagiotakopoulou V, Botsakis K, Delis F, Mourtzi T, Tzatzarakis MN, Dimopoulou A, PouliaN, Antoniou K, Stathopoulos GT, Matsokis N, Charalampopoulos I, Gravanis A,Angelatou F (2020) Anti-neuroinflammatory, protective effects of the syntheticmicroneurotrophin BNN-20 in the advanced dopaminergic neurodegeneration of "weaver" mice. Neuropharmacology 165:107919.
Pfisterer U, Kirkeby A, Torper O, Wood J, Nelander J, Dufour A, Björklund A, Lindvall O, Jakobsson J, Parmar M (2011) Direct conversion of human fibroblasts to dopaminergic neurons. Proc Natl Acad Sci U S A 108:10343-10348.
Qian H, Kang X, Hu J, Zhang D, Liang Z, Meng F, Zhang X, Xue Y, Maimon R, Dowdy SF,Devaraj NK, Zhou Z, Mobley WC, Cleveland DW, Fu XD (2020) Reversing a model of Parkinson's disease with in situ converted nigral neurons. Nature 582:550-556.
Qin H, Zhao AD, Sun ML, Ma K, Fu XB (2020) Direct conversion of human fibroblasts intodopaminergic neuron-like cells using small molecules and protein factors. Mil Med Res 7:52.
Rath A, Klein A, Papazoglou A, Pruszak J, Garcia J, Krause M, Maciaczyk J, Dunnett SB,Nikkhah G (2013) Survival and functional restoration of human fetal ventral mesencephalon following transplantation in a rat model of Parkinson's disease. Cell Transplant 22:1281-1293.
Rivetti di Val Cervo P, Romanov RA, Spigolon G, Masini D, Martín-Montañez E, Toledo EM, La Manno G, Feyder M, Pifl C, Ng YH, Sánchez SP, Linnarsson S, Wernig M, Harkany T, Fisone G, Arenas E (2017) Induction of functional dopamine neurons from human astrocytes in vitro and mouse astrocytes in a Parkinson's disease model. Nat Biotechnol 35:444-452.
Rosenblum S, Smith TN, Wang N, Chua JY, Westbroek E, Wang K, Guzman R (2015) BDNF pretreatment of human embryonic-derived neural stem cells improves cell survival and functional recovery after transplantation in hypoxic-ischemic stroke. Cell Transplant 24:2449-2461.
Sakata H, Niizuma K, Wakai T, Narasimhan P, Maier CM, Chan PH (2012a) Neural stem cells genetically modified to overexpress Cu/Zn-superoxide dismutase enhance amelioration of ischemic stroke in mice. Stroke 43:2423-2429.
Sakata H, Niizuma K, Yoshioka H, Kim GS, Jung JE, Katsu M, Narasimhan P, Maier CM, Nishiyama Y, Chan PH (2012b) Minocycline-preconditioned neural stem cells enhance neuroprotection after ischemic stroke in rats. J Neurosci 32:3462.
Samoylova EM, Baklaushev VP (2020) Cell reprogramming preserving epigenetic age: advantages and limitations. Biochemistry (Mosc) 85:1035-1047.
Schriebl K, Satianegara G, Hwang A, Tan HL, Fong WJ, Yang HH, Jungbauer A, Choo A (2011) Selective removal of undifferentiated human embryonic stem cells using magnetic activated cell sorting followed by a cytotoxic antibody. Tissue Eng Part A 18:899-909.
Shan X, Chi L, Bishop M, Luo C, Lien L, Zhang Z, Liu R (2006) Enhanced de novo neurogenesis and dopaminergic neurogenesis in the substantia nigra of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinson's disease-like mice. Stem Cells 24:1280-1287.
Sharma R, McMillan CR, Niles LP (2007) Neural stem cell transplantation and melatonintreatment in a 6-hydroxydopamine model of Parkinson's disease. J Pineal Res 43:245-254.
Shin JE, Jung K, Kim M, Hwang K, Lee H, Kim IS, Lee BH, Lee IS, Park KI (2018) Brain and spinal cord injury repair by implantation of human neural progenitor cells seeded onto polymer scaffolds. Exp Mol Med 50:1-18.
Shohayeb B, Diab M, Ahmed M, Ng DCH (2018) Factors that influence adult neurogenesis aspotential therapy. Transl Neurodegener 7:4.
Sirko S, Behrendt G, Johansson PA, Tripathi P, Costa M, Bek S, Heinrich C, Tiedt S, Colak D, Dichgans M, Fischer IR, Plesnila N, Staufenbiel M, Haass C, Snapyan M, Saghatelyan A, Tsai LH, Fischer A, Grobe K, Dimou L, et al. (2013) Reactive glia in the injured brain acquire stem cell properties in response to sonic hedgehog. [corrected]. Cell Stem Cell 12:426-439.
Sonntag KC, Song B, Lee N, Jung JH, Cha Y, Leblanc P, Neff C, Kong SW, Carter BS, Schweitzer J, Kim KS (2018) Pluripotent stem cell-based therapy for Parkinson's disease: Current status and future prospects. Prog Neurobiol 168:1-20.
Stricker SH, Götz M (2021) Epigenetic regulation of neural lineage elaboration: Implications for therapeutic reprogramming. Neurobiol Dis 148:105174.
Sun C, Ou X, Farley JM, Stockmeier C, Bigler S, Brinton RD, Wang JM (2012) Allopregnanolone increases the number of dopaminergic neurons in substantia nigra of a triple transgenic mouse model of Alzheimer's disease. Curr Alzheimer Res 9:473-480.
Sundberg M, Bogetofte H, Lawson T, Jansson J, Smith G, Astradsson A, Moore M, Osborn T, Cooper O, Spealman R, Hallett P, Isacson O (2013) Improved cell therapy protocols for Parkinson's disease based on differentiation efficiency and safety of hESC-, hiPSC-, and non-human primate iPSC-derived dopaminergic neurons. Stem Cells 31:1548-1562.
Tao Y, Vermilyea SC, Zammit M, Lu J, Olsen M, Metzger JM, Yao L, Chen Y, Phillips S, Holden JE, Bondarenko V, Block WF, Barnhart TE, Schultz-Darken N, Brunner K, Simmons H, Christian BT, Emborg ME, Zhang SC (2021) Autologous transplant therapy alleviates motor and depressive behaviors in parkinsonian monkeys. Nat Med 27:632-639.
Tay EXY, Chia K, Ong DST (2021) Epigenetic plasticity and redox regulation of neural stem cell state and fate. Free Radic Biol Med 170:116-130.
Thompson LH, Grealish S, Kirik D, Björklund A (2009) Reconstruction of the nigrostriatal dopamine pathway in the adult mouse brain. Eur J Neurosci 30:625-638.
Tran J, Anastacio H, Bardy C (2020) Genetic predispositions of Parkinson's disease revealed in patient-derived brain cells. NPJ Parkinsons Dis 6:8.
Trist BG, Hare DJ, Double KL (2019) Oxidative stress in the aging substantia nigra and the etiology of Parkinson's disease. Aging Cell 18:e13031.
Van den Berge SA, van Strien ME, Korecka JA, Dijkstra AA, Sluijs JA, Kooijman L, Eggers R, De Filippis L, Vescovi AL, Verhaagen J, van de Berg WD, Hol EM (2011) The proliferative capacity of the subventricular zone is maintained in the parkinsonian brain. Brain 134:3249-3263.
Van Kampen JM, Robertson HA (2005) A possible role for dopamine D3 receptor stimulation in the induction of neurogenesis in the adult rat substantia nigra. Neuroscience 136:381-386.
Volarevic V, Markovic BS, Gazdic M, Volarevic A, Jovicic N, Arsenijevic N, Armstrong L, Djonov V, Lako M, Stojkovic M (2018) Ethical and safety issues of stem cell-based therapy. Int J Med Sci 15:36-45.
Wakai T, Sakata H, Narasimhan P, Yoshioka H, Kinouchi H, Chan PH (2014) Transplantation of neural stem cells that overexpress SOD1 enhances amelioration of intracerebral hemorrhage in mice. J Cereb Blood Flow Metab 34:441-449.
Wang C, Liu F, Liu YY, Zhao CH, You Y, Wang L, Zhang J, Wei B, Ma T, Zhang Q, Zhang Y, ChenR, Song H, Yang Z (2011) Identification and characterization of neuroblasts in the subventricular zone and rostral migratory stream of the adult human brain. Cell Res 21:1534-1550.
Wang JM, Singh C, Liu L, Irwin RW, Chen S, Chung EJ, Thompson RF, Brinton RD (2010) Allopregnanolone reverses neurogenic and cognitive deficits in mouse model of Alzheimer's disease. Proc Natl Acad Sci U S A 107:6498-6503.
Wang S, Okun MS, Suslov O, Zheng T, McFarland NR, Vedam-Mai V, Foote KD, Roper SN, Yachnis AT, Siebzehnrubl FA, Steindler DA (2012) Neurogenic potential of progenitor cells isolated from postmortem human Parkinsonian brains. Brain Res 1464:61-72.
Wang S, Zou C, Fu L, Wang B, An J, Song G, Wu J, Tang X, Li M, Zhang J, Yue F, Zheng C, Chan P, Zhang YA, Chen Z (2015) Autologous iPSC-derived dopamine neuron transplantation in a nonhuman primate Parkinson's disease model. Cell Discov 1:15012.
Wang YK, Zhu WW, Wu MH, Wu YH, Liu ZX, Liang LM, Sheng C, Hao J, Wang L, Li W, Zhou Q, Hu BY (2018) Human clinical-grade parthenogenetic ESC-derived dopaminergic neurons recover locomotive defects of nonhuman primate models of Parkinson's disease. Stem Cell Rep 11:171-182.
Xiao Z, Lei T, Liu Y, Yang Y, Bi W, Du H (2021) The potential therapy with dental tissue-derived mesenchymal stem cells in Parkinson's disease. Stem Cell Res Ther 12:5.
Xie MQ, Chen ZC, Zhang P, Huang HJ, Wang TT, Ding YQ, Qi SS, Zhang C, Chen SX, Zhou P,Shao CC, Liao M, Sun CY (2017) Newborn dopaminergic neurons are associated with the migration and differentiation of SVZ-derived neural progenitors in a 6-hydroxydopamin-injected mouse model. Neuroscience 352:64-78.
Yoo J, Lee E, Kim HY, Youn DH, Jung J, Kim H, Chang Y, Lee W, Shin J, Baek S, Jang W, Jun W, Kim S, Hong J, Park HJ, Lengner CJ, Moh SH, Kwon Y, Kim J (2017) Electromagnetized gold nanoparticles mediate direct lineage reprogramming into induced dopamine neurons in vivo for Parkinson's disease therapy. Nat Nanotechnol 12:1006-1014.
Yoshihara M, Araki R, Kasama Y, Sunayama M, Abe M, Nishida K, Kawaji H, Hayashizaki Y, Murakawa Y (2017) Hotspots of de novo point mutations in induced pluripotent stem cells. Cell Rep 21:308-315.
Yoshimi K, Ren YR, Seki T, Yamada M, Ooizumi H, Onodera M, Saito Y, Murayama S, Okano H, Mizuno Y, Mochizuki H (2005) Possibility for neurogenesis in substantia nigra of arkinsonian brain. Ann Neurol 58:31-40.
Yu D, Ma M, Liu Z, Pi Z, Du X, Ren J, Qu X (2020) MOF-encapsulated nanozyme enhanced siRNA combo: Control neural stem cell differentiation and ameliorate cognitive impairments in Alzheimer's disease model. Biomaterials 255:120160.
Zenchak JR, Palmateer B, Dorka N, Brown TM, Wagner LM, Medendorp WE, Petersen ED, Prakash M, Hochgeschwender U (2020) Bioluminescence-driven optogenetic activation of transplanted neural precursor cells improves motor deficits in a Parkinson's disease mouse model. J Neurosci Res 98:458-468.
Zeng X, Couture LA (2013) Pluripotent stem cells for Parkinson's disease: progress and challenges. Stem Cell Res Ther 4:25.
Zhang P, Xie MQ, Ding YQ, Liao M, Qi SS, Chen SX, Gu QQ, Zhou P, Sun CY (2015) Allopregnanolone enhances the neurogenesis of midbrain dopaminergic neurons in APPswe/PSEN1 mice. Neuroscience 290:214-226.
Zhao M, Momma S, Delfani K, Carlén M, Cassidy RM, Johansson CB, Brismar H, Shupliakov O, Frisén J, Janson AM (2003) Evidence for neurogenesis in the adult mammalian substantia nigra. Proc Natl Acad Sci U S A 100:7925.
Zhou FC, Chiang YH (1995) Excitochemical-induced trophic bridging directs axonal growth of transplanted neurons to distal target. Cell Transplant 4:103-112.
Zhou H, Su J, Hu X, Zhou C, Li H, Chen Z, Xiao Q, Wang B, Wu W, Sun Y, Zhou Y, Tang C, Liu F, Wang L, Feng C, Liu M, Li S, Zhang Y, Xu H, Yao H, et al. (2020) Glia-to-neuron conversion by CRISPR-CasRx alleviates symptoms of neurological disease in mice. Cell 181:590-603.

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