Harnessing Neurogenesis for the Possible Treatment of Parkinson's Disease

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ABSTRACT

The discovery of neurogenesis in the adult brain has created new possibilities for therapeutics in neurodegenerative diseases. Neural precursor cells, which have been found in various parts of the brain, e.g., the subventricular zone (SVZ) and substantia nigra (SN), have promising potential to replace the extensive loss of neurons occurring in neurodegenerative disorders. In Parkinson's disease (PD) the degeneration of nigral dopaminergic neurons and consequently the nigrostriatal pathway, which has been found to innervate proximally to the SVZ, causes motor and cognitive impairments. There is strong evidence that neurogenesis is impaired in PD, which has been related to the nonmotor symptoms of the disease. Recent evidence supports that this impairment in neurogenesis is partially caused by the lack of dopamine in one of the adult neurogenic niches, the SVZ. Thus, restoring the dopaminergic pathway in PD patients may have implications not only for motor function improvement, but for other cognitive and autonomic symptoms. Currently, there are no effective treatments that can stop or reverse the neurodegeneration process in the brain. Here we review the neurogenic process and observed alterations found in PD animal models and postmortem brains of PD patients. Finally, we review several attempts to stimulate the neurogenic process for nigral and/or striatal dopaminergic restoration by transgenic expression, exercise, or cell therapy. J. Comp. Neurol. 522:2817–2830, 2014.

INDEXING TERMS: neurogenesis; Parkinson's disease; dopaminergic pathway

Adult neurogenesis, a process which new neurons are generated in the adult brain, was first discovered in rats more than 40 years ago (Altman, 1969). This concept was controversial for many years, until the late 1990s, when it was discovered in the human brain. Researchers injected a chemical marker for dividing cells into cancer patients and revealed newly generated cells in the hippocampus after postmortem histology (Eriksson et al., 1998). Today, the demonstration of neurogenesis and the isolation of neural precursor cells (NPCs) from the adult mammalian central nervous system (CNS) suggests new therapeutic possibilities for neurodegenerative diseases, in which there is extensive loss of cells in the brain.

NPCs exist in many regions throughout the adult brain (Zhao et al., 2008). However, they produce new neurons in only two specific regions under physiological conditions: the subventricular zone (SVZ) lining the lateral ventricles (Sanai et al., 2004) and the subgranular zone (SGZ) of the dentate gyrus (DG) in the hippocampus (Eriksson et al., 1998). The neurogenic process in the adult brain can be divided into a series of distinct developmental steps, which can be examined separately, and include the proliferation of precursor cells, the survival of newly born cells, the migration of these cells, differentiation into mature functional neurons, and finally, their integration in the neuronal network. The adult SVZ contains three main types of progenitor cells. Glial fibrillary acidic protein (GFAP)-positive, slowly dividing astrocyte-like-cells (type B) generate actively dividing transient amplifying cells (type C) which can be identified by the expression of the epidermal growth factor receptor (EGFR) (Hoglinger et al., 2004). C-cells differentiate into polysialic neural cell adhesion molecule+ (PSA-NCAM+)-restricted migrating neuroblasts (type A) (Zhao et al., 2008). The migrating neuroblasts migrate in chains ensheathed by the astrocytes (type B

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cells) to the olfactory bulb (OB) along the rostral migratory stream (RMS) (Kam et al., 2009). In the adult human brain, this migratory stream seems to be not as active as in rodents or young humans (Sanai et al., 2004; Wang et al., 2011). The transient amplifying cells (type C cells) form clusters next to the chains of migrating cells. After arrival in the center of the OB, neuroblasts detach from their chains and migrate in the overlying granular and periglomerular layers (Ming and Song, 2005). Most of the cells differentiate into gamma aminobutyric acid (GABA)-ergic granular neurons, and only small numbers differentiate into periglomerular neurons expressing tyrosine hydroxylase (TH) in addition to GABA (Winner et al., 2002; Kiyokage et al., 2010). Accordingly, SGZ contains: GFAP-expressing cells with radial processes (type 1) that generate GFAP-negative cells with short processes (type 2), which in turn give rise to doublecortin (DCX)-expressing neuroblasts (Zhao et al., 2008). Neurons born in the adult SGZ migrate into the granule cell layer of the dentate gyrus and become dentate granule neurons (Seri et al., 2004).

PARKINSON'S DISEASE

Parkinson's disease (PD) is the second most common neurodegenerative disorder, after Alzheimer's disease, affecting $\sim$1% of the population at the age of 65 years. It is a progressive, chronic disease involving many factors with a complex etiology. Its cardinal symptoms include motor symptoms such as bradykinesia, tremor, rigidity, and postural instability. Other manifestations are nonmotor-related PD symptoms, e.g., olfactory deficits, autonomic dysfunction, depression, cognitive deficits, and sleep disorders (Gaig and Tolosa, 2009).

Early studies have associated PD mainly with the degeneration of dopaminergic neurons of the substantia nigra (SN) pars compacta leading to loss of dopamine (DA) in the striatum, which is associated with the motor deficits of the disease (Koller et al., 1991). However, recent studies have revealed that the onset of the disease begins many years before the appearance of the motor symptoms and Braak et al. (2002) have proposed distinct stages of the disease progress in the brain (Gaig and Tolosa, 2009). PD is considered a synucleinopathy, the abnormal accumulation and aggregation of wildtype α-synuclein (α-syn) protein. It is a major component in intracellular inclusion bodies, termed Lewy bodies (LB), and argyrophilic processes, termed Lewy neurites (LN). Furthermore, studies have shown mechanisms of cell-to-cell propagation by alpha-synuclein oligomers released into the extracellular space and subsequent uptake of neighboring neurons (Danzer et al., 2009), astrocytes (Lee et al., 2010), as well as microglia (Alvarez-Erviti et al., 2011) which offer a possible explanation for the neuropathological stages proposed by Braak et al. (Olanow and Brundin, 2013). In cases with cognitive impairment, neuronal populations in the striatum, hippocampus, and neocortex are affected (Harding et al., 2002; McKeith et al., 2004). Accumulation of α-syn in these structures affects nonmotor functions such as neurogenesis and olfaction, contributing to PD pathology (Fleming et al., 2008; Desplats et al., 2012). Moreover, α-syn is hypothesized to activate the brain's innate immune system by the activation of microglia cells. Gao and Hong (2008) even suggested neuroinflammation as a driving force in the progression of DA degeneration. Consequently, neuroinflammation could directly affect neural stem cells and thereby inhibit the brain's endogenous regenerative potential (Das et al., 2011; Russo et al., 2011; Worlitzer et al., 2012).

At present, there are only symptomatic treatments for PD patients that offer relative relief for the motor symptoms. Dopamine replacement therapy—e.g., L-dopa, the gold standard of pharmacological therapy—does not effectively alleviate all PD features and has psychiatric side effects. Moreover, prolonged treatment causes loss of efficacy, dyskinesias, and nonmotor manifestations. Other treatments, such as deep brain stimulation, treat solely motor symptoms (Brichta et al., 2013). Cell replacement therapy (CRT) emerged three decades ago with the aim of replacing the lost DAergic neurons with new and healthy ones. As proof of concept it was demonstrated that by new DAergic neurons grafting, improvement in humans and animal models can be achieved. However, this approach still presents several technical and ethical problems which has prevented their clinical translation (Ganz et al., 2011). Through induced pluripotent stem cells (iPSC) technology, patient-specific stem cell lines were generated for disease modeling purposes and to treat patients with personalized, tissue-matched transplants, without the accompanying immunological complications (Park et al., 2008). It was demonstrated that DAergic neurons can be generated by mouse fibroblast-derived iPSCs (Wernig et al., 2008), PD patients somatic cells (Hargus et al., 2010), and more recently this was achieved through a 21-day fast protocol and 93% differentiation efficiency (Theka et al., 2013). All of the generated cells showed behavioral improvements after intrastriatal transplantation in the 6-OHDA PD animal model. Recently, it was demonstrated that ectopic expression of different sets of transcription factors can rapidly and efficiently convert mouse fibroblasts into functional DAergic neurons with therapeutic potential in animal models of PD (Kim

et al., 2011). iPSCs-DA and iDA represent a great advance for stem cell research and regenerative approaches in PD, holding tremendous potential for future clinical translation. However, they still have many drawbacks and further investigation is needed before clinical application is pursued. Although beneficial in research of the basic disease processes, patient-derived iPSCs and iNs may carry mutations, polymorphisms, or epigenetic marks that could make them more susceptible to develop PD-like features after transplantation (Thomson et al., 1998; Kordower et al., 2008; Arenas, 2010).

Although CRT is being studied extensively, a new approach by using endogenous neural stem cells (NSCs) in replacing degenerated neurons seems to be a very interesting strategy for repairing the damaged PD brain. By introducing factors to the PD-diseased brain, which will induce endogenously the generation of DAergic neurons and restoring the denervation of the striatum, and the SVZ eventually, may have beneficial effects on motor deficiencies and perhaps even cognitive impairments, without the technical complications of CRT implantation.

NEUROGENESIS IN PD MODELS

Many studies have found that in PD there are alterations in the neurogenic process. Neurogenesis is a complex mechanism which involves many factors, one of them being dopamine, as revealed by in vivo and in vitro studies (Berg et al., 2013).

DA neurons originating in the SN project to the SVZ in mice (Baker et al., 2004), primates (Freundlieb et al., 2006), and humans (Hoglinger et al., 2004), and create a dense innervation. Most cells surrounding these dopaminergic nerve endings have been shown to express the epidermal growth factor receptor (EGFR). EGFR is expressed by all C-cells and a small subset of B-cells, suggesting that the fibers project mainly to progenitor cells (Hoglinger et al., 2004). In addition, a number of studies revealed the presence of DA receptors, supporting the possible effect of DA on cells in the SVZ. D1-like receptors were detected in the cytoplasm of C-cells and in membranes of A-cells, while D2-like receptors were most abundant in C-cells but less so in A-cells in rats (Hoglinger et al., 2004).

The two main animal models of PD involve the specific destruction of SN dopaminergic neurons by neurotoxins, i.e., 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or 6-hydroxydopamine (6-OHDA) (Przedborski et al., 2000; Simola et al., 2007). Several studies have demonstrated that in an MPTP rodent model of PD there is reduced proliferation of progenitor cells in the SVZ, and a similar effect in the SGZ as well, resulting in reduced neurogenesis (Hoglinger et al., 2004; L'Episcopo et al., 2012).

Freundlieb et al. (2006) showed similar results in the SVZ of an MPTP model in nonhuman primates, while another investigation showed that no neurogenesis occurs at all in the striatum (Tande et al., 2006). In addition, unilateral administration of 6-OHDA into the nigrostriatal pathway causes a decline in the number of proliferating cell nuclear antigen (PCNA)-expressing cells. Proliferation was seen to be restored completely by selective agonists of D2-like receptors (Hoglinger et al., 2004), and more specifically the D3 dopamine receptor agonist (Van Kampen et al., 2004). However, Kippin et al. (2005) showed an increase in proliferation of NSCs on applying the chronic treatment of a D2 receptor antagonist. These results can be explained by the fact that while dopamine promotes proliferation of transient amplifying cells, it may inhibit stem cell proliferation. This inhibition may appear after chronic treatment, due to the fact NSCs divide less frequently than the amplifying cells (Berg et al., 2013). Baker et al., (2004) demonstrated a decrease in bromodeoxyuridine (BrdU)-labeled cells in the SVZ after 6-OHDA injection to the medial forebrain bundle and SN. However, this reduction in proliferative capacity has been challenged. MPTP-injected mice were labeled with markers for PCNA and phosphohistone-H3 to find that NSCs in the SVZ maintain their proliferative capacity (van den Berge et al., 2011). Other studies have reported an increase in precursor proliferation in the SVZ, but only a small increase in aged mice (Peng et al., 2008; Peng and Andersen, 2011).

Aggregation of human α-syn, in adult transgenic mice, resulted in reduced neurogenesis in the SVZ and DG, mainly because of diminished survival of NPCs in the neurogenic regions (Winner et al., 2002, 2004). Subsequently, they found that this decrease was mediated by a p53 repression of notch-1, as well as by increased induction of NPCs apoptosis (Desplats et al., 2012). Notch1 signaling has profound effects on the development of the nervous system, including maintenance of stem cell self-renewal, proliferation, neuronal differentiation, and glial determination (Louvi and Artavanis-Tsakonas, 2006).

NEUROGENESIS IN PD

Nowadays, studies of adult neurogenesis in PD use solely postmortem analyses, which are limited. Hoglinger et al. (2004) found that the dopaminergic innervation of EGFR+ subependymal zone cells is conserved in adult humans, and that in PD patients there is a

significantly reduced number of PCNA+ in the SVZ, in tissue fixation, compared with controls matched for age, gender, and time from death. Another study found a significantly reduced number of EGFR+ cells, which are C-cells, in the PD SVZ compared to matched controls (O'Keeffe et al., 2009b). Moreover, decreased EGF levels have been observed in the striatum and the prefrontal cortex of PD patients, possibly related to dopaminergic denervation (Iwakura et al., 2005). In addition, O'Sullivan et al. (2011) found that expression of Musashi1, a marker for NSCs and NPCs, correlates negatively with disease progression and positively correlated with the amount of lifetime levodopa used. These results provide strong evidence for the dopaminergic modulation of forebrain precursor proliferation in adult humans.

However, these results of reduced proliferation have been challenged by van den Berge et al. (2011), who showed that proliferation of NSCs in the SVZ, measured by PCNA and phospho-histone-H3, did not change in PD patients compared to matched controls. Furthermore, neurospheres were generated from the SVZ of PD patients, with a success rate similar to that of control donors, which indicates that the SVZ in PD still contains proliferative, multipotent neural stem cells (van den Berge et al., 2011).

These contradictory results may have several explanations. First of all, DA replacement treatment is only considered in O'Sullivan et al., which can cause variations in results. Different levels of DA is an important factor which may affect neurogenesis. Therefore, consideration of the DA replacement therapy and its characterization is necessary for precise analysis. Second, different cell quantification and analyzing methods are used, as discussed in van den Berge et al. (2012) and Hoglinger et al. (2012). Finally, there are variable postmortem delays, causing different levels of tissue deterioration. Interestingly, the generation of neurospheres in vitro implies that the SVZ maintains its proliferative capacity in PD but lacks the sufficient signaling for proliferation.

NEUROGENESIS IN THE SN: HEALTHY BRAIN AND PD MODELS

There have been several studies of the possibility of neurogenesis in the SN. Lie et al. (2002) have shown that new cells are born in the healthy SN. However, most of the newborn cells were associated with the glial progenitor marker NG2 and no mature neurons were detected. However, they have demonstrated that precursor cells isolated from the SN have the ability to differentiate into neurons in vitro (Lie et al., 2002). Another group has reported the generation of new mature nigral DA neurons under physiological conditions by colocalization of BrdU and TH (Zhao et al., 2003; Zhao and Janson Lang, 2009). However, Borta and Hoglinger (2007) raised the possibility that the markers seen represented cells that are adjacent, and not the same cell. A number of works have also described the expression of PSA-NCAM in the SN (Nomura et al., 2000; Yoshimi et al., 2005; Peng et al., 2008), a marker for multipotent progenitor cells in the SVZ and the DG and, additionally, is widely expressed in the mammalian brain (Xiong et al., 2008; Gomez-Climent et al., 2011; Bonfanti and Nacher, 2012). Borta et al. (2007) discussed that PSA-NCAM is expressed not only in neuroblasts, but in other cells undergoing plastic changes as well, and these results do not support the hypothesis of dopaminergic neurogenesis in the SN. Several studies have explored neurogenesis in the SN in PD models, and have not found newborn DA neurons (Kay and Blum, 2000; Lie et al., 2002; Cooper and Isacson, 2004; Frielingsdorf et al., 2004; Mohapel et al., 2005; Yoshimi et al., 2005). Other interesting findings involve the presence of low levels of oligodendrogenesis and astrogliogenesis in the SN after 6-OHDA lesion. However, the findings seem to be strongly dependent on the model and the timepoint of investigation (Kay and Blum, 2000; Lie et al., 2002; Yoshimi et al., 2005; Steiner et al., 2008; Klaissle et al., 2012). Nevertheless, increasing evidence suggests oligodendrogenesis as an interesting new potential therapeutic target in PD (Worlitzer et al., 2012).

Whether proliferation in the SN ultimately leads to the formation of mature DA neurons remains controversial, although in vitro and in vivo differentiation of SN precursors to neurons in the hippocampus imply that there may be a presence of neuronal differentiation inhibition or a lack of proneural signals in the local SN environment (Lie et al., 2002). Perhaps by inducing an appropriate environment with growth factors and DAergic differentiation signaling factors, DA-secreting cells can be generated. The conflicting results may be explained by variations in the labeling protocols. Recently, Zhao et al. (2009) proposed a sensitive protocol which involves higher doses of BrdU and an additional 3 weeks survival, to enable the maturation of the dividing cells into neurons at detectable levels. This implies the presence of dividing cells, although in smaller amounts and with slower division rates in the SN. However, even if in the future researchers will succeed in inducing DA neurogenesis in the SN, the more problematic issue is reinnervation of the striatum of these newborn cells.

USE OF EXOGENOUS FACTORS IN INDUCING DAERGIC NEUROGENESIS

As described above, the main pathology of PD is the degeneration of DA cells in the SN and denervation of the striatum. The presence of progenitor cells in the SVZ and SN of the parkinsonian brain has been demonstrated (Wang et al., 2012). Therefore, several studies have attempted to find factors which induce neurogenesis in these regions, to facilitate endogenous cell-replacement strategies (Table 1, Figure 1).

In several studies, transforming growth factor α (TGFα) has been introduced into the striatum of 6-OHDA-lesioned rats (Fallon et al., 2000; Cooper and Isacson, 2004; de Chevigny et al., 2008). All studies have shown increase in proliferation in the SVZ and migration of these cells to the DA-depleted striatum. However, only one study has found a small amount of newly generated dopaminergic neurons in the striatum, expressing TH and dopamine transporter (Kim et al., 2011). Moreover, they have shown a reversal of the motor dysfunction (Fallon et al., 2000). The other studies failed to demonstrate motor recovery and dopaminergic differentiation in the striatum, or in the SN. In addition, the increase in neurogenesis has not been found in naïve rats, implying a synergic effect of the lesion and TGFα (Cooper and Isacson, 2004; de Chevigny et al., 2008). It was found that the cells, which had migrated to the striatum, were of multipotent C-cell-phenotype, which in turn differentiated into mature neurons in the OB. This evidence suggests that such C-like cells require further midbrain or forebrain cues to differentiate into mature DA neurons suitable to improve function in PD (de Chevigny et al., 2008). Another study administered fibroblast growth factor 2 (FGF-2) to an MPTP model in mice (Peng et al., 2008). They found an increase in neuronal differentiation in the SVZ and the SN, but did not provide evidence of the cells eventually turning into dopaminergic neurons, and where the cells in the SN originated. Another group has shown that the addition of epidermal growth factor (EGF), to FGF-2, leads to an increase of proliferation and the number of neuroblasts in the SVZ of 6-OHDA-lesioned rats. This increase was more pronounced in the 6-OHDA-animals. However, most of the newly generated cells showed a glial phenotype (Winner et al., 2008). Increased proliferation in the SVZ and the striatum was seen in 6-OHDA rats, which received an intraventricular infusion of liver growth factor (LGF), and 25% of the proliferating cells were DCX-positive. Moreover, some of the newborn cells were mature neurons. However, these neurons have not been observed to be dopaminergic (Gonzalo-Gobernado et al., 2009). Other growth factors have been investigated. In a 6-OHDA model, platelet-derived growth factor (PDGF) and brain-derived neurotrophic factor (BDNF) have been shown to increase proliferation and differentiation into neurons in the striatum, yet they fail to induce dopaminergic neurons (Mohapel et al., 2005).

The effect of DA receptor (DAR) agonists on neurogenesis have been investigated. The chronic treatment of 7-OH-DPAT, a D3 receptor agonist, was administered to the third ventricle of 6-OHDA rats. This resulted in a rise in cell proliferation in the SVZ and SN, and more interestingly, an increase in the expression of TH in newborn cells in the SN. Moreover, these cells demonstrated functional recovery which lasted for at least 4 months after the treatment ended (Van Kampen and Eckman, 2006). Another study examined a combination of DAR agonists, for D1-like receptors and D2-like receptors, in a 6-OHDA model (O'Keeffe et al., 2009a). An increase in BrdU-positive cells in the SVZ and a significant increase of newborn neurons in the OB was demonstrated. Another DAR agonist, pramipexole (PPX), has been investigated and revealed an increase in the SVZ-OB system by increasing SVZ-cell proliferation and survival of newly generated neurons in the OB. However, no striatal neurogenesis has been noted (Winner et al., 2009).

A recent study investigated how cell proliferation and cell differentiation in the adult mouse SN would be altered after treatment with the antiinflammatory minocycline in the acute nigral 6-OHDA injection model of PD (Worlitzer et al., 2013). EdU+ cells in the SN were recorded, but there were no signs of new mature neurons 3 weeks after 6-OHDA injection. In this study, the low numbers of cells and the resulting high variations between animals made it difficult to make any assumptions about the effect of 6-OHDA-induced lesion and minocycline. Interestingly, Worlitzer et al. (2013) found a high percentage of DCX+ cells among the EdU+ cells in the SN. However, previous reports failed to demonstrate the presence of DCX+ cells in the SN (Frielingsdorf et al., 2004; Mohapel et al., 2005; Van Kampen and Eckman, 2006; Steiner et al., 2008; Klaissle et al., 2012). The explanation for these conflicting results may be due to the coimmunostaining of DCX with BrdU that often interferes with antibody binding of proteins with a low expression level. On the other hand, DCX expression has been linked to neural shape plasticity and growth cone dynamics (Burgess and Reiner, 2000; Nacher et al., 2001; Friocourt et al., 2003). Consequently, expression of DCX is not sufficient to prove the existence of neurogenesis in the SN. Another recent report detailed the effects of the injection of clustered ephrin-A1 into the lateral ventrical of 6-OHDA

TABLE 1.
Stimulation of Neurogenesis in Parkinson's Disease Models

Stimulus	Model	Main findings	DAergic neurogenesis	Behavioral improvement	Study
TGFα	6-OHDA in rats	Increase in SVZ proliferation, migration to striatum and neurogenesis	Yes In the striatum	Yes	Fallon et al., 2000
		Increase in SVZ proliferation, migration to striatum but no neuronal differentiation	No	No	Cooper and Isacson, 2004
		Increase in SVZ proliferation, migration to striatum but no neuronal differentiation	No	No	de Chevigny et al., 2008
FGF-2	MPTP in mice	Neurogenesis in the striatum and limitied neurogenesis in the SN	No	ND	Peng et al., 2008
FGF-2 & EGF	6-OHDA in rats	Increase in SVZ proliferation and increased number of neuroblasts in the SVZ and striatum. Gliogenesis rather than neurogenesis	No	ND	Winner et al., 2008
LGF	6-OHDA in rats	Increase in SVZ proliferation, migration to striatum and neurogenesis	No	No	Gonzalo-Gobernado et al., 2009
BDNF & PDGF	6-OHDA in rats	Increase in SVZ proliferation, migration to striatum or proliferation in the striatum and neurogenesis	No	ND	Mohapel et al., 2005
7-OH-DPAT (D3r agonist)	6-OHDA in rats	Increase in SVZ and SN proliferation, and neurogenesis in the SN	Yes In the SN	Yes	Van Kampen and Eckman, 2006
D1-like receptors & D2-like receptors agonists	6-OHDA in rats	Increase in SVZ proliferation Increase in neuronal differentiation in olfactory bulb	No	ND	O'Keeffe et al., 2009a
pramipexole (DAR agonist)	6-OHDA in rats	Increase in SVZ proliferation Increase in neuronal differentiation in OB	No	Yes	Winner et al., 2009
Minocycline	6-OHDA in mice	Increase in SN proliferation oligodendrogenesis and astrogliosis in the SN	No	ND	Worlitzer et al., 2013
Clustered ephrin-A1	6-OHDA in rats	Increase in SVZ proliferation migration to striatum, neurogenesis and angiogenesis Increased proliferation in the OB	Yes In the striatum	Yes	Jing et al., 2012
Exercise	MPTP in mice		ND	Yes	Fisher et al., 2004

TABLE 1. *Continued*

Stimulus	Model	Main findings	DAergic neurogenesis	Behavioral improvement	Study
		Down-regulation of DAT Up-regulation of the dopamine D_2 receptor			
		Decreased expression of striatal TH and DAT	No	Yes	Petzinger et al., 2007
		The number of DAergic cells in SN increased	Yes (but not known if new-born or recovered)	Yes	Smith et al., 2011)
	Healthy Aged mice	Increased neurogenesis in the hippocampus	ND	ND	Wu et al., 2008
Exercise	Healthy Aged mice	Oligodendrogenesis in the SN	No	ND	Klaissle et al., 2012
	MPTP in mice	Oligodendrogenesis in the SN only after levodopa			
Intrastriatal hMSCs transplant	6-OHDA in rats	Increase in SVZ proliferation, migration to striatum and neurogenesis	No	ND	Cova et al., 2010
Induced hMSCs transplantation in the SVZ	6-OHDA in rats	Increase in SVZ proliferation, migration to striatum and neurogenesis	No	Yes	Kan et al., unpublished
Systematic administration of hMSCs	MPTP in mice	Augmented neurogenesis in both the SVZ and SN, and increased DAergic differentiation in the SN	Yes (but not known if local or migrated)	ND	Park et al., 2012

DAergic, dopaminergic; ND, not demonstrated; 6-OHDA, 6-hydroxydopamine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; SN, substantia nigra; OB, olfactory bulb; SVZ, subventricular zone; TGFα, transforming growth factor α; FGF, fibroblast growth factor; EGF, epidermal growth factor; LGF, liver growth factor; PDGF, platelet-derived growth factor; BDNF, brain-derived neurotrophic factor; D3r, D3 receptor; DAT, dopamine transporter; hMSC, human mesenchymal stem cells.

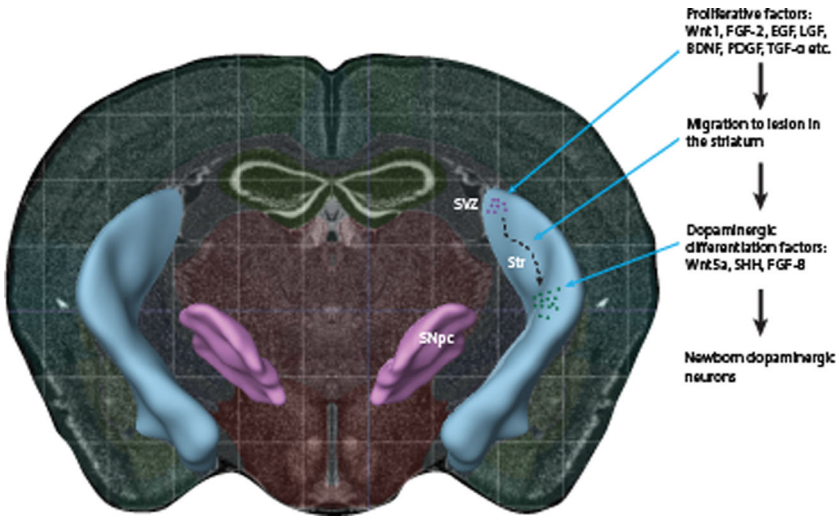

Figure 1. A schematic illustration of potential treatment for PD by enhancing endogenous dopaminergic neurogenesis. SVZ, subventricular zone; SNpc, substantia nigra pars compacta; St, striatum; FGF, fibroblast growth factor; BDNF, brain-derived neurotrophic factor; EGF, epidermal growth factor; LGF, liver growth factor; PDGF, platelet-derived growth factor; TGF-α, transforming growth factor α; SHH, sonic hedgehog.

rats. Proliferation in the SVZ increased, as did the number of BrdU+ cells in the striatum and OB, and the number of cells expressing TH in the striatum. In addition they also observed augmented angiogenesis (Jing et al., 2012).

These inconsistent results concerning neurogenesis in the SN and SVZ indicate that a thorough and in-depth study, investigating multiple factors which stimulate neuroprotection and/or DA neuroregeneration, is warranted. Moreover, studies which administer factors inducing DAergic differentiation in these areas are needed. For example, members of the Wnt family, i.e., Wnt1, 3a, and 5a, have been reported to play a major role in DA neuron generation and development, among other factors such as sonic hedgehog and FGF8 (reviewed extensively in Kim, 2011).

EXERCISE AND NEUROGENESIS IN PD MODELS

Motor performance is dependent on the interaction between automatic and cognitive control of movement (Mazzoni and Wexler, 2009; Redgrave et al., 2010). In PD, the preferential loss of DA neurons in the dorsal basal ganglia leads to increased cognitive control of motor movements to compensate for the diminished automatic control. As a result, individuals with PD have a much larger cognitive load in executing motor or cognitive tasks (Wu and Hallett, 2008; Redgrave et al., 2010). Studies in the last decade have demonstrated the role of exercise, involving both automatic and cognitive control of movement, in improving motor performance in PD.

Epidemiological studies have also supported a link between energetic exercise and reduced risk for PD (Chen et al., 2005; Xu et al., 2010). Exercise is described as a physical activity that is planned, structured, and repetitive for the purpose of conditioning any part of the body. The importance of exercise in PD, is that repeating and challenging the body while skill training, leads to the improvement of motor performance. Since prefrontal cognitive circuits are critically involved in early phases of motor learning, another important component of exercise in PD is cognitive engagement.

Animal models provide an important tool to investigate the mechanisms by which exercise induces neuroplasticity in the mammalian brain. A study has shown that treadmill exercise in mice led to increased motor performance. The exercise was started 5 days after acute MPTP administration, which allowed cell death to occur before exercise was initiated (Fisher et al., 2004). The results showed a downregulation of DAT, a protein important in regulating dopamine uptake, and upregulation of the DA D_2 receptor, a receptor important in motor behavior (Fisher et al., 2004). Another study found no difference in striatal DA levels between MPTP-treated mice with or without exercise. However, they did find increased striatal DA in saline-treated mice undergoing exercise (Petzinger et al., 2007). Additionally, immunohistochemical staining for striatal TH and DAT demonstrated decreased expression in MPTP-

treated mice that exercised, as compared to MPTP-treated mice that did not exercise (Petzinger et al., 2007). This suggests that the benefits of treadmill exercise, while beneficial for all mice, are different in MPTP-lesioned versus non-MPTP-treated mice. However, exercise did not result in DA neuron restoration in the SN. In another MPTP-model, exercise was initiated after 7 days of MPTP treatment (Smith et al., 2011). Exercise led to improvement in gait performance for both the mice who received MPTP and those who did not. Furthermore, the effects of early treadmill-based intervention appear to be unique in mice who received MPTP. The number of DA cells in SNpc increased after treadmill exercise (Smith et al., 2011). This can be explained by the moderate lesion in this study: there was a 50% decrease in TH-labeled neurons in the SNpc, compared to a severe loss, more than 60%, in others (Fisher et al., 2004; Petzinger et al., 2007). However, the authors did not examine whether the TH+ neurons were newborn or recovered neurons. Consequently, exercise may be more beneficial for patients with mild symptoms or at risk.

Neurogenesis has been shown to increase in the hippocampus of middle-aged mice, which had undergone 5 weeks of treadmill training. The researchers observed the enhancement of NSC proliferation, the promotion of neurite growth of newborn neurons, and the increased survival of newborn neurons (Wu et al., 2008). A different study demonstrated that physical activity and enriched environment induced generation of newborn cells in the adult mouse SN. The majority of these cells expressed NG2, a marker for oligodendrocytic precursor cells. Mature oligodendrocytes have been found as well in the SN. However, this effect was seen in MPTP-mice only after levodopa treatment, which implies the role of dopamine oligodendrogenesis. NG2+ cells have been reported to bear neuroprotective and neuroregenerative capacities in the adult (Klaissle et al., 2012).

In conclusion, physical exercise has proven to enhance neurogenesis in healthy mice, improve motor function in PD models, and to help restore SN damage in a moderate PD model. However, there are very few studies which examined the connection between exercise, neurogenesis, and their effect on motor or cognitive performance. Thus, more studies linking exercise and neurogenesis enhancement in PD are needed to further elucidate this subject.

MESENCHYMAL STEM CELL-BASED NEUROGENESIS INDUCTION IN PD MODELS

Mesenchymal stem cells or mesenchymal stromal cells (MSCs), first identified by Friedenstein et al. (1974), are multipotent cells with a self-renewal capacity that can differentiate into cells of several distinct mesodermal lineages, including bone, cartilage, and adipose tissues (Pittenger et al., 1999). They are fibroblast-like cells and can be isolated from almost all tissues, including bone marrow, muscle, fat, dermis, tooth, placenta, amniotic membrane and fluid, endometrium, umbilical cord and cord blood, lung, liver, spleen, tonsils, peripheral blood, and blood vessels (Bianco et al., 2008). A neural predisposition hypothesis of MSCs was proposed by the findings that MSCs express the basal level of neural genes, including nestin, neuron-specific enolase, and beta-tubulin III (Blondheim et al., 2006) and that MSCs can be induced to differentiate into neural-like cells under similar culture conditions for neural induction of embryonic stem cells (Sanchez-Ramos et al., 2000; Woodbury et al., 2000; Levy et al., 2003; Khoo et al., 2008). This neural plasticity aroused great interest for the therapeutic application of MSCs for various neurodegenerative diseases (NDDs). However, neuronal and dopaminergic differentiation of bone-marrow-derived cells requires transdifferentiation from a mesodermal to a neuroectodermal lineage. This possibility is still being debated (Bertani et al., 2005; Phinney and Prockop, 2007; Tondreau et al., 2008). Other characteristics that make MSCs an interesting possibility for cell therapy in NDD are their homing capabilities to the damaged tissue via circulation, immunosuppression qualities (Prockop and Oh, 2012) to protect further tissue damage and secretion of factors that induce neuroprotection and endogenous repair.

It has been demonstrated that both intravenous and intracerebral injection of MSCs improved functional recovery and reduced lesion size in rodents in ischemic brain injury models. Different administration methods resulted in similar outcomes, implying that the mode of administration was not a key factor in recovery (Jackson et al., 2010). Migration of MSCs to neuronal lesions and increased viability were also demonstrated in a 6-OHDA animal model (Hellmann et al., 2006). It is still unknown how and why MSCs home to damaged tissues but studies imply inflammation as one probable cause (Hong et al., 2009; Yagi et al., 2010). This ability may ease the administration of MSCs in clinical applications.

MSCs transplanted into the injury site have been shown to affect neurogenesis. A study which transplanted hMSCs intrastriatally in a 6-OHDA rodent model demonstrated increased proliferation and transformation of progenitor cells into neuroblasts (Type A), which migrated towards the lesioned striatum. However, the enhancement of neurogenesis could not be detected in

sham animals following transplantation, suggesting a synergistic action between the MSCs and the lesion on neurogenesis (Cova et al., 2010). We also reported an increase in proliferation and differentiation of neuronal progenitors in the SVZ of healthy mice after MSCs transplantation in the SVZ. The NPCs expressed NeuN 3 weeks later. It was hypothesized that secretion of trophic factors from the transplanted cells caused this increase (Kan et al., 2011). A continuing investigation demonstrated that the transplantation of induced-MSCs to the SVZ in a 6-OHDA rodent model stimulated endogenous astrogenesis and showed neuroprotection of DA terminals leading to improved gait performance (Kan et al., unpubl.).

A recent study has demonstrated that hMSC administration significantly augmented neurogenesis in both the SVZ and SN of MPTP animal model, which led to increased differentiation of NPCs into dopaminergic neurons in the SN. However, their data (Park et al., 2012) did not provide information as to whether hMSC-induced cell proliferation in the SN occurred from resident NPCs or cells that migrated from the SVZ. Moreover, hMSC administration notably increased the expression of EGFR in the SVZ of MPTP-treated PD animals, and coculture of hMSCs significantly raised the release of EGF in the medium of MPP+-treated NPCs (Park et al., 2012). EGF is known to be an endogenous regulator of SVZ neurogenesis, leading to the significant enhancement in the proliferation and migration of NPCs in the SVZ (Kuhn et al., 1997).

The use of MSCs for therapeutics in PD seems encouraging, exhibiting immunomodulatory, neuroprotective, and neuroregenerative properties. Furthermore, MSCs raise no ethical concerns and can be administered by autologous transplantation (Kan et al., 2007). Moreover, the probability of MSCs being tumorigenic is low and they have been shown to be safe in clinical trials (Venkataramana et al., 2010).

CONCLUSION

The research on neurogenesis in the adult brain has just begun to make progress in the field of neurorestoration and neuroprotection. Only recently it was discovered that NPCs exist in many regions in the adult human brain, including the SN, and they can be isolated and differentiated in vitro. Moreover, these cells exist in the brain of PD patients as well, although it seems that there is a decrease in their proliferation, seemingly from the dopaminergic denervation. PD-associated pathology not only has an impact on the degeneration of mature neurons but also influences the generation of neural progenitor populations in the adult brain. Therefore, stimulation of the endogenous stem and progenitor cell population might be a promising means to restore some of the diseased regions in PD. There is no concrete evidence as to whether there is dopaminergic neurogenesis in PD or PD models. Studies show promising evidence that exogenous factors increase neurogenesis, and may even stimulate dopaminergic neurogenesis in the SVZ and the SN. However, in our opinion, a combination of multiple factors, one which induces proliferation and another inducing DAergic differentiation, is needed to succeed in dopaminergic restoration and further studies are required to find the ideal "mix" of factors. The striatum seems a more feasible target for this DAergic restoration, because of the unlikely reinnervation of the SVZ of newborn DAergic neurons in the SN. Exercise seems to be beneficial in improving the motor performance in PD, although a direct correlation to neurogenesis has not yet been proven. The use of MSCs for the treatment of PD shows promise, but it is mostly neuroprotective and not restorative, which is most needed. Further investigation still needs to be conducted to clarify the precise mechanisms and effects these cells have on neurogenesis, and will it lead to restore dopamine levels in the brain. Moreover, iPSCs is attracting more and more interest in procedures of neurorestoration, pushing aside MSCs.

The variability in PD animal models leads to contradictory results, warranting the need for a standardized model to imitate the disease progression in humans and incorporate additional pathologies other than the loss of the striata-nigra pathway, perhaps lesioning of the pathway in transgenic α-syn mice. Finally, developing new ways of in vivo imaging of adult neurogenesis in PD patients and animal models may provide much more insight into how we see it today.

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CONFLICT OF INTEREST

D.O. and E.M. are consultants in Brainstorm Cell Therapeutics, Israel. The other authors declare no conflict of interest.

ROLE OF AUTHORS

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Analysis

and interpretation of data: O.L, J.G., D.O., Drafting of the article: O.L, J.G. Critical revision of the article for important intellectual content: E.M., D.O. Obtained funding: D.O.

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