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Review

Neurogenesis in Neurotoxin-induced Animal Models for Parkinson's Disease—A Review of the Current Status

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Abstract: Animal models for Parkinson's disease (PD) are essential for understanding its pathogenesis and for development and testing of new therapies. Discoveries of endogenous neurogenesis in the adult mammalian brain give new insight into the cell-based approach for treatment of neurodegenerative disorders, such as PD. Although a great deal of interest has been focused on endogenous neurogenesis in neurotoxin-induced animal models for PD, it still remains controversial whether neural stem cells migrate into the injured area and contribute to repopulation of depleted dopaminergic neurons in neurotoxin-injured adult brains. The purpose of this review is to examine the data available regarding neurogenesis in neurotoxin-induced animal models of PD. It is hoped that data from the animal investigations available in the literature will promote understanding of the neurotoxin-induced animal models for PD. (J Toxicol Pathol 2009; **22**: 101–108)

Key words: animal model, Parkinson's disease, neurogenesis, MPTP, 6-OHDA

Introduction

Parkinson's disease (PD) is an age-related degenerative disorder of the central nervous system. It is often characterized by muscle rigidity, tremor, slowing of physical movement (bradykinesia) and, in extreme cases, loss of physical movement (akinesia). The symptoms of PD are attributed to the loss of pigmented dopamine-secreting (dopaminergic) neurons in the pars compacta region of the substantia nigra (SN) and a subsequent striatal deficiency of dopamine. Idiopathic PD is also pathologically characterized by the presence of cytoplasmic neuronal inclusions, called Lewy bodies, in the affected region of the brain. The etiology of the disease has been poorly understood, and development of novel non-dopaminergic therapeutic strategies has remmained challenging since initial description of the disease by James Parkinson in 1817[1]. Although pharmacological dopamine replacement strategies provide temporary symptomatic relief, there are at present no therapeutic methods for halting progressive neuronal cell loss[2].

Animal models are an important tool in elucidating mechanisms involved in the pathological process and in investigating new therapeutic strategies for PD. Currently, both genetic and toxic models of PD are available, but use of neurotoxins, such as 6-hydroxydopamine (6-OHDA), paraquat, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and rotenone, is still the most popular means of modeling destruction of the nigrostriatal dopaminergic neurons seen in PD[3]. Among these neurotoxins, 6-OHDA and MPTP have been more extensively used by investigators to produce PD models despite their limitations. The neurotoxin 6-OHDA is a structural analogue of catecholamines, dopamine and noradrenaline, and exerts its toxic effects on catecholaminergic neurons[4]. Since 6-OHDA does not penetrate the blood brain barrier in adult rats, it must be administrated stereotactically into the substantia nigra or striatum to damage the dopaminergic nigrostriatal system[5, 6]. Recognition of MPTP as a neurotoxin occurred early in 1982, when several young drug addicts mysteriously developed a profound Parkinsonian syndrome after intravenous use of street preparations of meperidine analogs that, unknown to anyone, were contaminated with MPTP[7, 8], an incidental byproduct during the chemical synthesis of a meperidine analog (Fig. 1) with potent heroin-like effects[7]. MPTP has been found to produce irreversible Parkinsonism in humans almost indistinguishable from PD[8, 9]. It is also well known that MPTP depletes striatal dopamine and causes damage to the substantia nigra pars compacta (SNpc) dopaminergic neurons in non-human primates and several species of rodents[10–13].

Recently, a great deal of interest has been focused on

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stem cell therapies for PD. In addition to fetal nigral transplantation, the capability of self-repair in the central nervous system (CNS) in the adult mammalian has also given a new perspective in the cell-based approach for treatment of neurodegenerative disorders. In PD or its animal models, much attention has been attracted to whether the depletion of dopaminergic neurons triggers activation of neural stem cells and their subsequent migration into the damaged area, finally resulting in repopulation of dopaminergic neurons in the SN. This review will provide the body of evidence available for endogenous neurogenesis in response to neurotoxin damage in animal models of PD.

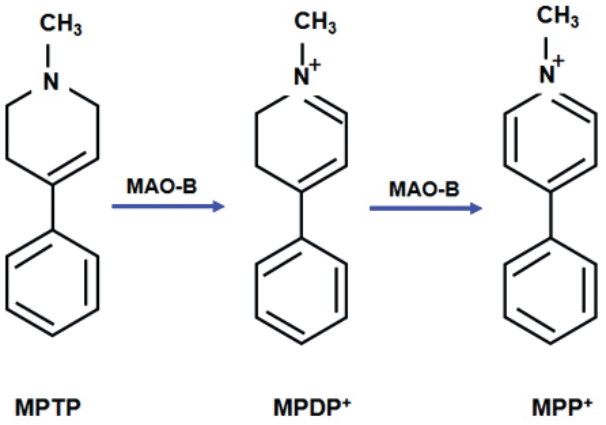

Fig. 1. MPTP is a byproduct during the chemical synthesis of a meperidine analog. MPTP neurotoxicity develops only after metabolization to MPDP+ by MAO-B in glial cells and then to MPP+, the active toxic compound.

The Origin and Properties of Neural Stem Cells

Neurogenesis continues into adult life in the brains of rodents[14, 15], nonhuman primates[16, 17], and humans[18, 19]. It is confined largely to two discrete areas, the subventricular zone (SVZ) and the subgranular zone (SGZ) of dentate gyrus (DG)[20] (Fig. 2).

The SVZ, located throughout the inner wall of the lateral ventricle, is the largest germinal region and harbors neural stem cells that retain the capacity to generate multiple cell types[21]. The SVZ is composed of neural progenitor cells (migrating neuroblasts, A cells), neural stem cells (astrocytes, B cells), neural precursor cells (rapidly dividing transit amplifying cells, C cells) and ependymal cells[22–24] (Fig. 2). Neural stem B cells divide to give rise to clusters of precursor C cells, which in turn generate neuroblast A cells[23]. Newly generated A cells in the SVZ migrate through a network of tangential pathways in the lateral wall of the lateral ventricle and then converge onto the rostral migratory stream (RMS) to enter the olfactory bulb (OB) via a "chain migration" behavior[25], in which they differentiate into

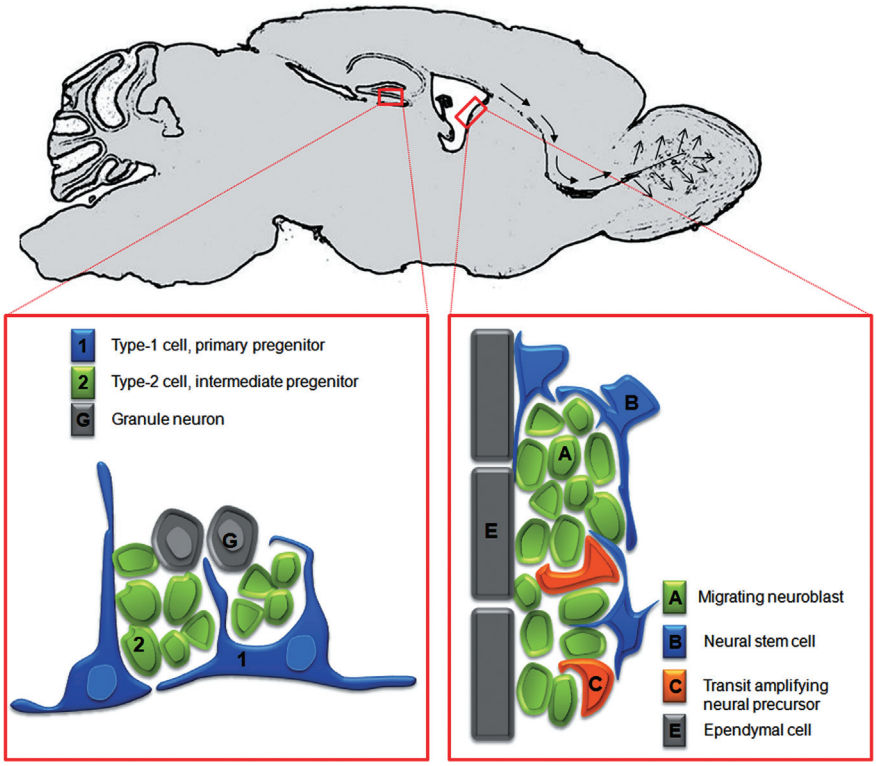

Fig. 2. Schematic representations of the migratory pattern of newly generated A cells in the SVZ (up) and the cell populations in the SGZ of the DG (bottom left) and SVZ (bottom right) in the adult rodent brain (adapted from Ref. 24).

granule cells and interneurons (Fig. 2)[25, 26]. It has been suggested that Prokineticin 2 (PK2) serves as a chemoattractant for SVZ-derived neuroblast A cells, which appears to guide the migration of A cells from the SVZ through the RMS to their final layers in the OB[27]. The multipotential A cells residing in the SVZ and RMS are eliminated through apoptosis to maintain a balance for proper development of the mammalian nervous system[28–30]. Metalloproteinases (MMPs) may also play a crucial role in the migration of individual A cells since their migration rates are reduced by the presence of inhibitors of MMPs[31]. The architecture and function of the adult human SVZ differs significantly from that described in other mammals. There are four layers of varying thicknesses and cell densities throughout the lateral ventricular wall: a monolayer of ependymal cells (Layer I), a hypocellular gap (Layer II), a ribbon of cells (Layer III) composed of astrocytes and a transitional zone (Layer IV) into the brain parenchyma[32].

The DG is a part of the hippocampal formation. Neuronal progenitors in the adult mammalian hippocampal DG reside in the SGZ, which is located in the hilus immediately beneath the granular layer of the DG[33, 34]. Neurogenesis in the DG was first demonstrated 40 years ago by autoradiography in rodents[35] and thereafter was further demonstrated in all mammalian species including human[19] and nonhuman primates[36, 37]. Two types of neural progenitors can be identified in the SGZ according to their specific morphologies and expression of unique sets of molecular markers[38] (Fig.2). The primary progenitors (type-1 cells) have the appearance of radial glia, which also express glial fibrillary acidic protein (GFAP). They share similar features with type the B cells residing in the adult SVZ and are suggested to be a putative stem cell population[39]. Type-2 cells (intermediate progenitors) are GFAP-negative and highly proliferative[40], sharing similar features with the neuroblast A cells residing in the adult SVZ. Type-2 cells may arise from type 1 cells, but direct evidence delineating this lineage relationship is still lacking[38]. Newborn type-2 cells disperse and migrate a short distance into the granule cell layer where they differentiate, extend axons and express neuronal marker proteins[34, 41]. The migration of newborn neurons in the dentate gyrus may also be controlled by guidance cues, as these cells only migrate to the hilus or molecular layer under pathological conditions, such as in animal models of temporal lobe epilepsy[38].

Dopamine and Neurogenesis

Dopamine plays important roles in many physiological functions, including motor control, mood and the reward pathway[42], and as a neurotransmitter, it binds to the five types of dopamine receptor, D1, D2, D3, D4 and D5, and their variants. The D1 and D5 receptors are members of the D1-like family (D1L), whereas the D2, D3 and D4 receptors are members of the D2-like family (D2L). It is known that both PD and neurotoxin-induced PD animal models are mainly characterized by a hallmark of dopamine depletion. Furthermore, dopamine replacement therapy is the most effective treatment for PD to date. Herein, the involvement of dopamine in adult neurogenesis is discussed.

Neurogenesis is a key event during both physiological and pathological processes and is regulated by a variety of stimuli, such as hormones, intrinsic growth factors, neurotransmitters, exogenously applied agents, environmental factors, exercise and age.

Dopamine has been shown to regulate cell cycles in the developing brain[43]. The activation of dopamine receptors influences cell proliferation in the lateral ganglionic eminence (LGE) and the neuroepithelium of the frontal cortex in embryonic mice[44, 45]. Dopamine has attracted a lot of attention concerning the role it plays in adult neurogenesis in recent years. In PD animal models, the first thing that needs to be clarified is whether decreased/increased neurogenesis is associated with the dopamine-involved signaling pathway or is a response to destruction of the nigrostriatal system.

By using immunohistochemical and ultrastrctural analyses, Höglinger *et al*. have provided anatomical evidence that D2L receptors in the SVZ are expressed predominately on C cells, whereas A cells express both D1L and D2L receptors in the mouse brain[46]. Dopaminergic fiber has also been confirmed to contact precursor cells in the SVZs of adult humans and primates[46, 47]. Thus, dopamine seems to play a key role in the regulation of adult mammalian neurogenesis. Findings from *in vitro* experiments show that activation of the D2L receptor directly stimulates proliferation of SVZ precursor cells[46]. This is supported by a recent study, in which stimulation of dopamine D2 receptors increased the proliferation of neural progenitor cells both *in vivo* and *in vitro*[47]. The depletion of nigrostriatal dopamine reduces precursor cell production in the SVZs of mice and aged primates and further leads to impaired neurogenesis in the OB[46, 47]. Based on the finding that acute administration of SCH23390, a D1 receptor antagonist, reduces the number of 5'-bromodeoxyuridine (BrdU)-positive cells in the SGZ of the DG, Suzuki *et al*. suggest that adult neurogenesis in the DG may be regulated naturally by dopamine via D1-like receptors[48]. A very recent report demonstrated that dopamine regulates adult neurogenesis by a mechanism of modulating ciliary neurotrophic factor (CNTF) expression in SVZ astrocytes[49]. The findings of Van Kampen *et al*. indicate that administration of the dopamine D3 receptor agonist, 7-hydroxy-N,N-di-n-propyl-2-aminotetralin (7-OH-DPAT), significantly increases the proliferation of neural stem cells (NSCs) in the adult rat brain[50, 51] but not in the adult mouse brain[51]. Furthermore, chronic intraventricular administration of 7-OH-DPAT triggers a profound induction of cell proliferation in the rat SN and promotes adoption of a neuronal phenotype in some of these newly generated cells[52].

On the other hand, it has been suggested that treatment with 7-OH-DPAT does not affect the proliferation, survival or neurogenesis of murine and human neural progenitor cells derived from the fetal midbrain *in vitro*[53]. Moreover,

chronic treatment with the antipsychotic drug haloperidol leads to increased NSC numbers, resulting in more progenitors and more new neurons and glia in the adult rat brain due to a mechanism of antagonizing dopamine at the D2 receptors on NSCs[54]. Treatment with haloperidol also increases dentate granule cell proliferation in the gerbil hippocampus[55, 56]. These controversial data question the involvement of dopamine in adult neurogenesis.

Neurogenesis in MPTP Animal Models for PD

MPTP neurotoxicity develops only after metabolization to 1-methyl-4-phenyl-2,3-dihydropyridinium (MPDP+) by an enzyme, monoamine oxidase (MAO)-B, and further to 1-Methyl-4-phenylpyridinium (MPP+), the active toxic compound (Fig. 1)[7]. Since MPP+ is selectively transported into presynaptic dopaminergic nerve terminals through the dopamine transporter (DAT)[57–61] and the absence of the DAT on dopaminergic neurons confers complete protection against MPTP toxicity[59, 60], the neurotoxicity of MPTP is suggested to be selective to dopaminergic neurons in the SN. MPTP is mainly used in non-human primates and rodents[62, 63], and the latter are less sensitive to MPTP neurotoxicity[64]. In rodents, C57BL/6 mice have been used in a great deal of reports for creation of a PD animal model for understanding the disease pathogenesis, despite their limitions.

Zhao *et al*. first provided morphological evidence that administration of MPTP leads to a 2-fold increase of BrdU incorporation in nigral dopaminergic neurons[65]. Newly generated dopaminergic neurons has been demonstrated to be derived from the cells lining the ventricular system[65]. By using the nestin second intron enhancer-controlled LacZ reporter transgenic mouse model coupled with the MPTP lesion system, Shan *et al*. demonstrated that there is increased dopaminergic neurogenesis in the SN[66], supporting the findings of Zhao *et al*. as described above. In rat and macaque monkey hemi-Parkinsonian models treated stereotatically with MPP+, the dopamine-depleted hemisphere showed more polysialic acid (PSA)-positive cells, candidates for newly differentiated young neurons, than the intact side, and a small number of tyrosine hydroxylase (TH)-positive cells were demonstrated to be PSA-positive[67].

In the striatum of the MPTP-treated aged macaque, although there is an increase in the number of TH-immunoreactive neurons, the increase has been suggested to be derived from pre-existing GABAergic interneurons (phenotype shift), not neurogenesis[68]. Yamada *et al*.[69] performed a retroviral vector-based method to evaluate neurogenesis in the OBs of MPTP-treated mice, and the results showed that dopaminergic neurogenesis can be enhanced in the OB after dopaminergic neuron loss. However, their findings do not clearly indicate relevance to the damaged nigrostriatal system. Increased neurogenesis has also been confirmed in a very recent paper[70], in which MPTP lesions increased the incorporation of BrdU as well as the number of cells that co-expressed BrdU and the immature neuronal marker doublecortin (DCX) in the DG, SVZ and striatum, but not in the SN of the MPTP-treated mouse, although the differentiation of newly generated cells into dopaminergic neurons was not investigated.

On the other hand, decreased neurogenesis has also been demonstrated in MPTP animals. Höglinger *et al*. demonstrated that proliferation of transit amplifying cells (C cells) is impaired in the SVZ and SGZ in the MPTP mouse model for PD[46]. C cells are the target of a dopaminergic innervations, and impaired neurogenesis in the SVZ is thus suggested to be mediated by MPTP administration-induced dopamine depletion[46]. Furthermore, reduced C cells lead to a subsequent decrease in A cells[46]. In adult macaques, MPTP-induced dopamine depletion also results in decreases in the number of proliferating cell nuclear antigen-positive (PCNA) cells and A cells in the SVZ[47], suggesting that intact dopaminergic nigro-subventricular innervation is crucial for sustained neurogenesis in aged primates[47].

In recent years, the selective neurotoxicity of MPTP in the dopaminergic system of the adult brain has been challenged. A number of studies[71–73] have demonstrated that MPTP also destroys forebrain migrating neuroblasts and nigrostriatal glial cells (astrocytes) in the adult mouse brain. In our previous studies[72, 73], the number of apoptotic cells in the SVZ and RMS peaked at 24 hours after MPTP injections and decreased thereafter, paralleling the changes in number of cleaved caspase-3-positive cells. The cells undergoing apoptosis in the SVZ, RMS and OB were identified as A cells using immunohistochemistry and ultrastructural analyses, while a few were astrocytes (B cells), and none were transit-amplifying procursors (C cells)[72, 73]. The decrease in A cell numbers was most marked on day 2 and lasted to day 8 after administration[72, 73]. We also demonstrated that MAO-B inhibitors, such as deprenyl or N-(2-aminoethyl)-4-chlorobenzamide (Ro 16-6491) completely protected A cells against MPTP neurotoxicity, suggesting that MPTP neurotoxicity in A cells is also mediated by the conversion of MPTP into MPP+ by MAO-B[74].

A cells are main populations in the SVZ and RMS of the adult brain, and proliferation of A cells may contribute to dult endogenous neurogenesis. Using BrdU labeling, we provided evidence that MPTP injury leads to transiently impaired neurogenesis in the adult mouse SVZ and OB. The majority of BrdU-positive cells in the SVZ are rapidly depleted by MPTP, and only a few BrdU-positive cells migrate into the OB thereafter (Fig. 3). Thus, impaired neurogenesis is more likely to be due to an MPTP-induced decrease in A cells rather than a mechanism through dopamine depletion as reported in previous studies[46, 47].

Impaired neurogenesis persists for about two weeks in the MPTP-injured mouse brain and is followed by recruitment of cells in the SVZ (He *et al*., unpublished data). The self-repairing process for the damaged germinal region is evidenced by regeneration of A cells (He *et al*., unpublished data). Thus, neurogenesis in the nigrostriatal system in response to MPTP damage, if it does occur, may

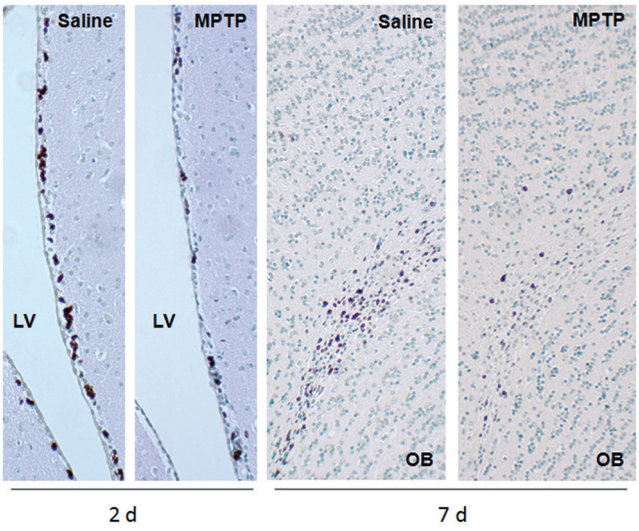

Fig. 3. MPTP reduces the number of BrdU+ cells in the SVZ and OB. The majority of BrdU-positive cells in the SVZ are rapidly depleted at 2 days, and only few BrdU-positive cells migrate into the OB at 7 days after MPTP administration.

be a later event in the self-repairing process of the injured SVZ. In a recent study[70], increases in BrdU-positive cells and BrdU-Dcx double-labeled cells in the SVZ were observed 14 d after acute MPTP treatment. This, however, may have been due to an activated self-repairing process in the SVZ rather than a response to nigrostriatal damage.

Neurogenesis in 6-OHDA Animal Models for PD

Since 6-OHDA does not penetrate the blood brain barrier in adults, it must be administrated stereotactically into the substantia nigra or striatum to damage the dopaminergic nigrostriatal system[5, 6]. Although administration of 6-OHDA produces a decreased striatal dopamine level and loss of dopaminergic neurons in the SN in the adult brain, the 6-OHDA model does not mimic any clinical or pathological features of PD. In adult rats, when the right mesencephalic dopaminergic neurons are ablated by stereotactical injection of 6-OHDA into the right nigrostriatal pathway, the number of PCNA-positive cells in the SVZ ipsilateral to the lesion decreases[46]. Impaired proliferation of cells in the SVZ has also been suggested to be mediated by a mechanism of dopamine depletion as suggested in MPTP animals[46]. Evidence from C57BL/6 mice injected unilaterally with 6-OHDA into the midbrain part of the medial forebrain bundle also show impaired neurogenesis, and 6-OHDA-induced dopaminergic denervation of the striatum reduces CNTF mRNA levels in the SVZ, which further contributes to negative regulation of the proliferation of cells[49].

Although the proliferation of NSCs in the SVZ and DG in the adult brain is an important neurogenesis process and has been demonstrated in some neurodegenerative diseases, it remains controversial whether those proliferated NSCs migrate into the damaged area and repopulate the lost functional neurons.

Treatment of 6-OHDA also leads to a transient decrease in neurogenesis in the olfactory granule cell layer, but, in contrast, an increase of neurogenesis is present in the glomerular layer[75]. The increased neurogenesis in the glomerular layer is characterized by more newly generated neuronal nuclear antigen (NeuN)- and TH-expressing neurons, indicating a shift in cell fate decision for newly generated neuronal precursors targeted at the glomerular layer[75].

Frielingsdorf *et al*. detected increases in cell proliferation in the SN in rats injected with 6-OHDA into the median forebrain bundle; however, none of the newly born cells expressed a dopaminergic phenotype, and there is no evidence of neural stem cells emanating from the cerebroventricular system and migrating to the substantia nigra[76]. Growth factors, such as platelet-derived growth factor (PDGF-BB) and brain-derived neurotrophic factor (BDNF), can induce striatal neurogenesis in adult rats with 6-OHDA lesions, and there are no indications of any newly born cells differentiating into dopaminergic neurons following growth factor treatment both in the striatum and in the SN[75]. Surprisingly, in a salamander 6-OHDA model for PD, robust and complete regeneration of the mesencephalic and diencephalic dopamine system occurs after elimination of dopaminergic neurons[77]. This regeneration leads to histological restoration and full recovery of motor behavior[77]. This 6-OHDA salamander model gives new insight into the animal models for PD and may be helpful for understanding the molecular mechanism of dopaminergic neurogenesis.

The findings of Steiner *et al*. indicate that the absolute numbers of newborn cells in the SN are not affected by dopamine depletion in the 6-OHDA rat model for PD. Instead, 6-OHDA lesions induce a specific downregulation of generation of newborn nigral astrocytic cells[78].

Conclusion

The replacement of lost neurons in a damaged brain area is the best direction for therapeutic development in relation to neurodegenerative diseases. Based on all of the available evidence, neurogenesis is still a most controversial issue in studies of neurodegenerative diseases and their animal models. In MPTP animal models for PD, we have demonstrated that MPTP destroys A cells in the SVZ and OB in the adult brain and then transiently impairs neurogenesis; however, it remains to be investigated whether and how multiple potential neural stem cells migrate into the nigrostriatal system and replace the lost dopaminergic neurons. With increased attention to dopaminergic neurogenesis in neurotoxin-induced animal models for PD, it is expected that the models will become useful as tools for understanding the mechanisms for neurogenesis in PD and that many questions regarding neurogenesis in

neurodegenerative diseases will be resolved.

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