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FIBROBLAST GROWTH FACTOR 2 ENHANCES STRIATAL AND NIGRAL NEUROGENESIS IN THE ACUTE 1-METHYL-4-PHENYL-1,2,3, 6-TETRAHYDROPYRIDINE MODEL OF PARKINSON'S DISEASE

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Abstract

In response to injury, endogenous precursors in the adult brain can proliferate and generate new neurons, which may have the capacity to replace dysfunctional or dead cells. Although injury-induced neurogenesis has been demonstrated in animal models of stroke, Alzheimer's disease (AD) and Huntington's disease (HD), studies of Parkinson's disease (PD) have produced conflicting results. In this study, we investigated the ability of adult mice to generate new neurons in response to the parkinsonian toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which causes selective degeneration of nigrostriatal dopamine neurons. MPTP lesions increased the incorporation of 5-bromo-2'-deoxyuridine-5'-monophosphate (BrdU), as well as the number of cells that co-expressed BrdU and the immature neuronal marker doublecortin (DCX), in two neuroproliferative regions—the subgranular zone of the dentate gyrus (DG) and the rostral subventricular zone (SVZ). BrdU-labeled, DCX-expressing cells were not found in the substantia nigra (SN) of MPTP-treated mice, where neuronal cell bodies are destroyed, but were present in increased numbers in the striatum, where SN neurons lost in PD normally project. Fibroblast growth factor-2 (FGF-2), which enhances neurogenesis in a mouse model of HD, also increased the number of BrdU/DCX-immunopositive cells in the SN of MPTP-treated mice. Thus, MPTP-induced brain injury increases striatal neurogenesis and, in combination with FGF-2 treatment, also stimulates neurogenesis in SN.

Keywords

fibroblast growth factor; Parkinson's disease; proliferation; progenitor; striatum; substantia nigra

Parkinson's disease (PD) is the second most frequently occurring neurodegenerative disorder after Alzheimer's disease (AD), affecting about 1% of the population over the age of 50 in North America (Forno, 1996; Lang and Lozano, 1998). Despite progress in understanding molecular mechanisms in PD, fully effective treatment remains elusive. One new potential strategy for replacing midbrain dopaminergic neurons in PD is based on endogenous neuroproliferation in the rostral subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus (DG). Neurogenesis is increased in these regions in certain neurological disorders including AD (Jin et al., 2004b), Huntington's disease (HD) (Curtis et al., 2003) and stroke (Jin et al., 2001). Furthermore, endogenous neurogenesis in neuroproliferative zones of the adult brain is enhanced by administration or overexpression of growth factors, including brain-derived neurotrophic factor (BDNF) (Pencea et al., 2001), epidermal growth factor (EGF) (Craig et al., 1996), fibroblast growth factor 2 (FGF-2) (Kuhn

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et al., 1997; Wagner et al., 1999), heparin-binding EGF (HB-EGF) (Jin et al., 2002b), stem cell factor (SCF) (Jin et al., 2002a) and vascular endothelial growth factor (Jin et al., 2002c). Growth factors such as insulin-like growth factor-I (IGF-I) (Lichtenwalner et al., 2001) and HB-EGF (Jin et al., 2003a) also stimulate neurogenesis in the aged brain.

Conflicting results have been obtained regarding whether animal models of PD stimulate endogenous neurogenesis, as reported for animal models of certain other neurodegenerative disorders. Rat substantia nigra (SN) has been reported to contain neuronal progenitor cells, identified by labeling with bromodeoxyuridine (BrdU) and expression of immature or mature neuronal lineage markers (Lie et al., 2002). Mouse SN has also been reported to contain a small number of BrdU-positive, dopaminergic cells thought to originate in the SVZ (Zhao et al., 2003). However, other laboratories have reported that they were unable to replicate these findings, calling into question the ability of endogenous neurogenesis in the adult to generate SN dopaminergic neurons, at least in the absence of disease-related stimulation (Frielingsdorf et al., 2004). The discordant results of studies to date suggest that the relationship between neurogenesis and Parkinsonism may be complex and that differences in the animal models employed or in the severity or duration of disease may explain some of the disparities.

In this study, we investigated the effect of acute 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride (MPTP) administration on neurogenesis in the adult mouse brain, using BrdU to label proliferating cells and cell type-specific antibodies to characterize their phenotype.

EXPERIMENTAL PROCEDURES

MPTP and FGF-2 administration

MPTP (20 mg/kg free base, Sigma, St. Louis, MO, USA) dissolved in saline was administered intraperitoneally to male C57BL/6 mice (8-week-old, Charles River Laboratories, Wilmington, MA, USA) every 2 h for four doses. Mice used as controls received an equivalent volume of saline. Following the last MPTP injection, mice were injected intraperitoneally with either PBS alone or recombinant human FGF-2 (38 μ g/kg, Chemicon, Temecula, CA, USA) in PBS daily for 10 days (days 0–10 after MPTP or saline administration). Experimental protocols were in accordance with the National Institutes of Health Guidelines for Use of Live Animals and were approved by the Animal Care and Use Committee at the Buck Institute. All possible efforts were made to minimize the number of animals used and their suffering.

BrdU administration

BrdU (50 mg/kg; Sigma) dissolved in saline was given intraperitoneally twice daily, at 8-h intervals, on consecutive days (days 1–3, 7–9, or 1–14 after MPTP or saline administration). Mice were killed on day 14 or 21 after MPTP or saline administration. This schedule labels cells undergoing DNA replication over 3 or 14-day spans ending 2 or 3 weeks after MPTP administration.

BrdU-immunopositive cell counting

Brains were removed after perfusion with saline and 4% paraformaldehyde in phosphate-buffered saline (PBS). Adjacent 50- μ m sections were cut with a cryostat and stored at -80°C . Sections were pretreated with 50% formamide, 280 mM NaCl, and 30 mM sodium citrate at 65°C for 2 h, incubated in 2 M HCl at 37°C for 30 min, and rinsed in 0.1 M boric acid, pH 8.5, at room temperature for 10 min. Sections were incubated in 1% H_2O_2 in PBS for 15 min, in blocking solution (2% goat serum, 0.3% Triton X-100, and 0.1% bovine serum albumin in PBS) for 2 h at room temperature, and with 2 μ g/ml of mouse monoclonal anti-BrdU antibody (Roche) at 4°C overnight. Sections were washed with PBS, incubated with biotinylated goat anti-mouse secondary antibody (Vector, Burlingame, CA, USA; 1:200) for 2 h at 25°C ,

washed, and placed in avidin–peroxidase conjugate (Vector) solution for 1 h. The horseradish peroxidase reaction was detected with 0.05% diaminobenzidine (DAB) and 0.03% H₂O₂. Processing was stopped with H₂O, and sections were dehydrated through graded alcohols, cleared in xylene, and cover-slipped in permanent mounting medium (Vector).

BrdU-positive cells in SGZ and SVZ were counted blindly in five DAB-stained, 50- μ m coronal sections per mouse, spaced 200 μ m apart. Cells were counted under high-power (200 $\times$) on a Nikon E300 microscope with a Magnifire digital camera and the image was displayed on a computer monitor. Results were expressed as the average number of BrdU-positive cells per section.

Fluorescence immunohistochemistry

Sections were fixed with 4% paraformaldehyde in PBS for 1 h at room temperature, washed twice with PBS, and incubated in 2 M HCl at 37 °C for 1 h. After washing again, sections were incubated with blocking solution, then with primary antibodies at 4 °C overnight, and with secondary antibodies in blocking solution at room temperature for 2 h. The primary antibodies used were mouse monoclonal anti-BrdU (Roche, Indianapolis, IN, USA; 2 μ g/ml), sheep polyclonal anti-BrdU (Bioscience Resource Project, Sacramento, CA, USA; 25 μ g/ml), rabbit anti-Ki-67 antigen (Zymed, South San Francisco, CA, USA; 1:100), mouse monoclonal anti-neuronal nuclear antigen (NeuN) (Chemicon; 1:200), affinity-purified goat polyclonal anti-doublecortin (DCX) (Santa Cruz Biotechnology, Santa Cruz, CA, USA; 1:200), rabbit polyclonal anti-TUC-4 (Chemicon; 1:1000), mouse monoclonal anti- β III-tubulin (Caltag Laboratories, Burlingame, CA, USA; 1:400), mouse monoclonal anti-glial fibrillary acidic protein (GFAP) (Sigma; 1:400), rat anti-mouse CD11b (Serotec Inc., Raleigh, NC, USA; 1:50), mouse monoclonal anti-2',3'-cyclic nucleotide 3'-phosphodiesterase (CNPS) (Chemicon; 1:500), rat anti-polysialic acid-neural cell adhesion molecule (PSA-NCAM) (BD Biosciences, San Jose, CA, USA; 1:100), and rabbit polyclonal anti-tyrosine hydroxylase (TH) (Chemicon; 1:200). The secondary antibodies were Alexa Fluor 488- or 594-conjugated donkey anti-sheep, anti-mouse, anti-rat, anti-goat, or anti-rabbit IgG (Molecular Probes, Carlsbad, CA, USA; 1:200). Fluorescence signals were detected with an LSM 510 NLO Confocal Scanning System mounted on an Axiovert 200 inverted microscope (Carl Zeiss Ltd., Thornwood, NY, USA) equipped with a two-photon Chameleon laser (Coherent Inc., Santa Clara, CA, USA). Three-color images were scanned using Argon and 543 HeNe lasers. IMARIS (Bitplane AG, St. Paul, MN, USA) imaging software was used for three-dimensional image reconstruction. Images were acquired using LSM 510 Imaging Software (Carl Zeiss Ltd.) as described previously (Peng et al., 2007). BrdU single-labeled cells, and cells double labeled for BrdU and specific markers, were counted in three 50- μ m coronal sections per mouse, spaced 200 μ m apart. Controls included omitting or preabsorbing primary or omitting secondary antibodies.

Statistical analysis

All data are expressed as mean $\pm$ S.E. for the number (*n*) of mice per group. Differences among the means for all experiments described were analyzed using two-way ANOVA with time or treatment as the independent factor. Newman-Keuls post hoc test was employed when differences were observed by analysis of variance testing ($P<0.05$).

RESULTS

Neurogenesis in the DG and rostral SVZ is increased following acute MPTP administration

Because neurogenesis persists in the adult mammalian brain and can be regulated by physiological and pathological events, we investigated its possible involvement in the brain's response to MPTP-induced neurotoxicity. Mice received MPTP (four i.p. injections per day of 20 mg/kg body weight at 2-h intervals) and proliferating cells were labeled with BrdU (two

injections per day of 50 mg/kg body weight at 8-h intervals) over 3-day periods, beginning on day 1 or 7 following the last MPTP injection. Mice were killed at 14 days after the lesion. Acute MPTP administration increased the incorporation of BrdU into cells in two neuroproliferative regions—the SGZ of the DG and the rostral SVZ (Fig. 1). To quantify changes in BrdU labeling following MPTP injection, we counted the number of BrdU-reactive nuclei in brain sections from saline- vs. MPTP-injected mice. As shown in Fig. 1, acute MPTP administration resulted in approximately 80% and 60% increases in the numbers of BrdU-labeled cells in the DG (control, days 1–3, 83.1 ± 3.5 ; days 7–9, 79.9 ± 4.2) and SVZ (control, days 1–3, 140.1 ± 15.5 ; days 7–9, 172.9 ± 18.9), respectively, as compared with saline-injected controls at 14 days. The number of BrdU-labeled cells with days 7–9 schedule was significant higher than days 1–3 schedule in the SVZ of MPTP-treated mice ($P < 0.05$), whereas there was no significant difference between days 1–3 and days 7–9 schedules on BrdU incorporation in the DG of MPTP-treated mice (Fig. 1). This difference is most likely due to MPTP-induced neurodegeneration. MPTP administration can cause nigral cell loss in mice, as documented by a large decrease in TH-positive neurons in the SN and TH-positive fibers in the striatum at 7 days after the last injection (Jackson-Lewis and Przedborski, 2007).

Relationship between BrdU labeling and DCX immunoreactivity

To investigate whether BrdU labeling following acute MPTP challenge correlates with labeling of neuronal precursors in the proliferation zones, we double-labeled brain sections with antibodies against BrdU and the developmentally-regulated marker DCX, a microtubule-associated protein found in the soma and processes of newborn and migrating neurons during development (Gleeson et al., 1999) and in lesion-induced adult neurogenesis (Magavi et al., 2000). Compared with saline controls, there were more BrdU-labeled cells expressing DCX in both the DG and the SVZ of the MPTP-injured brains (Fig. 2). BrdU-labeled cells co-expressed both DCX and proliferating cell nuclear antigen (not shown) suggesting that they were nascent neurons. BrdU/DCX-positive cell counts in the DG (control, days 1–3, 21.7 ± 2.9 ; days 7–9, 20.2 ± 3.1) and SVZ (control, days 1–3, 56.1 ± 3.5 ; days 7–9, 62.5 ± 5.2) were approximately 150–180% and 50–80% higher, respectively, in MPTP-treated mice compared with saline-treated mice at 14 days after the lesion (Fig. 2).

Acute MPTP induces neurogenesis in the striatum and the SN

Although pathological processes can enhance neurogenesis in the adult brain, the fate of the newborn neurons that are produced and their role in brain repair are not well understood. To determine whether acute MPTP-induced neuronal proliferation is associated with migration of nascent neurons from proliferation zones toward the injury site, we labeled proliferating cells by daily injections of BrdU for 14 consecutive days and mapped the migration of cells labeled by cell proliferation markers and antibodies against neuronal marker proteins for up to 21 days following the last MPTP administration. We used DCX, a microtubule-associated protein associated with migrating neurons (Gleeson et al., 1999), as the principal marker of an immature neuronal phenotype. DCX appears to be important for the normal developmental migration of cortical neurons, because mutations in DCX in humans lead to syndromes characterized by migrational arrest of these neurons and manifested clinically by lissencephaly, subcortical laminar heterotopia, mental retardation, and seizures (des Portes et al., 1998). In the normal adult brain, DCX is expressed in the SVZ and rostral migratory stream (RMS), but only in rare, single cells in the striatum. We found a similar pattern of expression in our saline-treated mice, whereas DCX-labeled cells were abundant in the striatum of MPTP-treated mice (Fig. 3A). As shown in Fig. 3B, DCX/BrdU-positive cell counts in striatum were fivefold higher in MPTP-treated mice than in saline-treated mice at 21 days after the lesion, suggesting that new neurons arose in or migrated to the striatum. To characterize the cells labeled by DCX after MPTP lesioning, we performed quadruple-labeling with antibodies against BrdU, DCX, and two neuronal lineage makers: β III-tubulin and TOAD/Ulip/CRMP family protein 4

(TUC-4). Markers of new neurons, including both β III-tubulin (Fig. 3C) and TUC-4 (Fig. 3D), were detected in cells that also expressed DCX.

Because neuronal stem or progenitor cells from the adult SN can give rise to neurons after transplantation (Lie et al., 2002), we hypothesized that MPTP-induced death of dopaminergic neurons in the SN might stimulate endogenous neurogenesis in this region. Sections were screened for newly generated neurons or astrocytes by staining for (a) BrdU or the cell-cycle marker Ki67 (not shown), (b) neuronal or glial markers (NeuN, β III-tubulin, CNPS, CD11b, GFAP, PSA-NCAM) and (c) a dopaminergic marker (TH). Although BrdU co-localized with the immature neuronal marker (embryonic) PSA-NCAM in some cells, none of the newly generated cells in SN expressed NeuN, β III-tubulin, CNPS, CD11b, GFAP or TH in the SN. Furthermore, triple-label studies showed that PSA-NCAM/BrdU-immunopositive cells did not coexpress the mature neuronal marker NeuN or the dopaminergic marker TH in the SN.

FGF-2 stimulates neurogenesis in MPTP-treated brains

FGF-2 has been shown to stimulate the proliferation, differentiation, and survival of cells of neuronal lineage. More specifically, FGF-2 promotes neurogenesis in a transgenic mouse model of HD (Jin et al., 2005). To test whether FGF-2 can stimulate neurogenesis following MPTP treatment as well, FGF-2 was injected intraperitoneally for 10 days and BrdU was given for 14 days following acute MPTP administration, and mice were killed 1 week later. As shown in Fig. 4A, the number of BrdU-immunoreactive cells in the SVZ increased significantly after FGF-2 administration, especially in MPTP-treated mice. BrdU-immunopositive cells co-expressed DCX in the SN, suggesting that FGF-2 can increase the number of newborn neurons in the SN following MPTP-induced injury. Under no conditions did we observe any cells immunoreactive for both DCX and BrdU in the SN of vehicle-treated control mice (Fig. 4B). In addition, FGF-2 treatment significantly increased the numbers of TUC-4/BrdU-positive and β III-tubulin/BrdU-positive cells in the striatum and PSA-NCAM/BrdU-positive cells in the SN (Fig. 4), confirming that the number of new neurons was increased. Taken together, these data suggest that FGF-2 stimulates neuronal proliferation and differentiation in adult MPTP-treated brains.

DISCUSSION

In this study, we investigated the effect of acute MPTP administration on neurogenesis in the adult mouse brain, using BrdU to label proliferating cells and cell type-specific antibodies to characterize their phenotype. The results indicate that acute MPTP treatment promotes neurogenesis in the SGZ, SVZ and striatum, and, after administration of FGF-2, in the SN. Therefore, MPTP-induced Parkinsonism appears to stimulate neurogenesis in a manner that could contribute to functional replacement of nigrostriatal circuitry, especially in the presence of FGF-2.

We report here that acute administration of the neurotoxin MPTP, which produces a syndrome that resembles human PD, stimulates neurogenesis in the adult mouse brain. Neurogenesis was identified by the occurrence of BrdU labeling within individual cells, suggesting recent provenance, and by the expression of DCX and other neuronal markers, establishing neuronal lineage. In addition to its effect on DG and SVZ neurogenesis, MPTP increased the number of new neurons in the striatum and, following FGF-2 treatment, in the SN. Whether these striatal and nigral neurons arose from local progenitors or migrated from DG or SVZ cannot be resolved by the present data. However, the SVZ is immediately adjacent to the striatum and appears to provide the new neurons that migrate there in animal models of other cerebral disorders including stroke (Arvidsson et al., 2002; Jin et al., 2003b) and HD (Jin et al., 2005).

Previous work exploring neurogenesis in PD has produced conflicting results. As to whether endogenous neurogenesis occurs in the normal adult SN, the adult rat SN has been reported by one group to contain progenitor cells, identified by labeling with BrdU, that give rise exclusively to glia *in situ*; however, when cultured in the presence of FGF-2 or FGF-8 *in vitro* or transplanted into the dentate hilus *in vivo*, these progenitors produced cells that expressed immature (β III-tubulin) or mature (NeuN) neuronal markers (Lie et al., 2002). Another group has reported that the mouse SN contains a small number of TH-expressing cells that could be labeled with BrdU, and which were thought to originate in the SVZ because they could be labeled by intraventricular injection of the fluorescent tracer dye, 1,1'-diiodo-3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) (Zhao et al., 2003). However, other researchers reported that TH and BrdU were present in adjacent rather than the same SN cells and that DiI reached the SN by retrograde transport along the nigrostriatal tract (Frielingsdorf et al., 2004).

Conflicting results have also been obtained regarding whether animal models of PD, like animal models of other neurodegenerative disorders, stimulate endogenous neurogenesis. After unilateral injection of 6-hydroxydopamine (6-OHDA) into the rat medial forebrain bundle, no BrdU-positive cells that expressed β III-tubulin, NeuN or TH could be detected (Lie et al., 2002). In contrast, administration of 6-OHDA into the SN and ventral tegmental area combined with intrastriatal infusion of transforming growth factor- α (TGF- α) led to expansion of the EGF receptor-positive cell population in the rat SVZ and migration of these cells toward the site of TGF- α infusion (Fallon et al., 2000). Moreover, some of these migrating cells could be labeled with BrdU and expressed immature (β III-tubulin, DCX) or dopaminergic (TH, DA transporter) neuronal markers. Following a single s.c. dose of MPTP, the number of TH-positive nigral cells that incorporated BrdU was reported to be increased (Zhao et al., 2003). Nevertheless, no such change was observed when 6-OHDA was injected into the medial forebrain bundle with or without concomitant administration of BDNF (Frielingsdorf et al., 2004) or TGF- α (Cooper and Isacson, 2004), and the previous result was attributed to failure to distinguish adjacent BrdU-positive and TH-positive cells by three-dimensional confocal analysis (Frielingsdorf et al., 2004).

In some respects, our results also contrast with previous findings using the MPTP model (Zhao et al., 2003). In our study, most BrdU-labeled cells in the SN failed to express markers for microglia, astrocytes, or neurons, although some expressed the neuronal lineage marker PSA-NCAM. These findings suggest that only limited neurogenesis may occur in SN of acutely MPTP-treated mice. Therefore, if significant injury-induced neurogenesis occurs following acute MPTP administration, it must occur outside SN (e.g. in the SVZ), and the new neurons produced either fail to migrate to the SN, or do so too slowly to be detected within the time course over which these experiments were conducted.

Our results indicate that, in contrast to the absence of evidence for large-scale neurogenesis in the SN, MPTP-induced neurogenesis contributes new neurons to the striatum. These could arise locally, or could migrate from elsewhere, such as SVZ. This resembles findings in a mouse model of HD (Jin et al., 2005), although the primary sites of pathology are different in the two disorders. In fact, our observations in acutely MPTP-treated mice are reminiscent of neurotransplantation strategies for PD in which the new cells are placed in the striatum rather than SN. Perhaps in both cases, the function of lost nigrostriatal cells can be restored, at least partly, by intrastriatal surrogates. In other neurodegenerative disorders, including stroke, AD and HD, neurogenesis is associated with directed migration to the site of injury. In PD, however, the new cells seem to be directed elsewhere (to the striatum rather than the SN). Perhaps this indicates that it is the degenerating nigrostriatal nerve terminals, situated in the striatum, which provide direction to newborn SVZ neurons. A neurogenesis signal emanating from degenerating nerve terminals would be consistent with the earlier involvement of terminals

than somata in MPTP toxicity (Kay and Blum, 2000). The idea that nerve-terminal rather than cell-body dysfunction might be the driving force for injury-directed neuromigration is also consistent with findings in a mouse model of AD (Jin et al., 2004a). There, increased neurogenesis is observed early in the course of the disease, when synaptic dysfunction and synaptic loss are present, but cell death cannot be demonstrated. In that disorder, too, it may be affected nerve terminals rather than cell bodies that provide the stimulus for neurogenesis and the migrational target for newborn neurons.

We found previously that basal neurogenesis was not appreciably altered in R6/2 HD transgenic mice, but that if these mice were treated with FGF-2, the number of new neurons in the affected striatum was increased about five-fold more than in FGF-2-treated wild type mice (Jin et al., 2005). Thus, the R6/2 HD mutation affects neurogenesis differently than do models of stroke (Jin et al., 2001) or AD (Jin et al., 2004a), in which basal neurogenesis is increased. In this respect, mouse models of HD and PD demonstrate similar results. Another similarity is that in both cases, FGF-2 stimulates neurogenesis at the principal site of cell loss. FGF-2 is expressed in both striatal and nigral neurons (Gonzalez et al., 1995), and loss of either could therefore produce a state of local FGF-2 deficiency that precludes a neurogenesis response to injury. This would be consistent with the finding that stroke-induced neurogenesis is reduced in FGF-2 knockout mice and restored by i.c.v. administration of an FGF-2-expressing herpes simplex virus amplicon vector (Yoshimura et al., 2001). In fact, FGF-2 is depleted from the SN in PD (Tooyama et al., 1993) and treatment with FGF-2 enhances histological and biochemical recovery from MPTP lesioning in mice (Date et al., 1993). Additional studies involving more prolonged monitoring and tests of neuronal function are required to evaluate these possibilities.

CONCLUSION

We conclude that, because new neurons were found at the principal site of MPTP-induced neuronal loss (SN) and in the major region to which these neurons normally project (striatum), increased neurogenesis in this model may represent a mechanism directed toward the replacement of dead or damaged neurons. If so, measures that further stimulate neurogenesis, such as the administration of neurogenesis-promoting drugs or growth factors, might have therapeutic potential in patients with PD.

Acknowledgments

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Abbreviations

AD	Alzheimer's disease
BDNF	brain-derived neurotrophic factor
BrdU	bromodeoxyuridine
CNPS	2',3'-cyclic nucleotide 3'-phosphodiesterase
DAB	diaminobenzidine
DCX	doublecortin
DG	dentate gyrus
DiI	1,1'-dioctadecyl-3,3,3',3'-tetramethylindolyl-carbocyanine perchlorate
EGF	epidermal growth factor
FGF-2	fibroblast growth factor 2

GFAP	glial fibrillary acidic protein
HB-EGF	heparin-binding epidermal growth factor
HD	Huntington's disease
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride
NeuN	neuronal nuclear antigen
PBS	phosphate-buffered saline
PD	Parkinson's disease
PSA-NCAM	polysialic acid-neural cell adhesion molecule
SGZ	subgranular zone
SN	substantia nigra
SVZ	subventricular zone
TGF- α	transforming growth factor- α
TH	tyrosine hydroxylase
TUC-4	TOAD/Ulip/CRMP family protein 4
6-OHDA	6-hydroxydopamine

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Fig. 1.

BrdU incorporation in DG and SVZ of MPTP-treated mice. Left, Representative sections showing BrdU-positive cells in the DG (A) and SVZ (B). Scale bar=50 μ m. Right, Quantitative comparison of BrdU-positive cells in the DG (A) and SVZ (B) at 14 days after MPTP lesioning. White bars, saline-treated; black bars, MPTP-treated. Data are mean $\pm$ S.E., $n=5$. * $P<0.01$, ** $P<0.001$ compared with saline-treated (Newman-Keuls post hoc test).

Fig. 2.

Relationship between BrdU incorporation and DCX expression in DG and SVZ. Left, Colocalization (yellow) of BrdU (red) and DCX (green) in the DG (A) and SVZ (B). Scale bar=50 μ m. Right, DCX/BrdU-positive cell counts in the DG (A) and SVZ (B) at 14 days after MPTP lesioning. White bars, saline-treated; black bars, MPTP-treated. Data are mean $\pm$ S.E., $n=5$. * $P<0.01$, ** $P<0.001$ compared with saline-treated (Newman-Keuls post hoc test). For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

Fig. 3. Neurogenesis in striatum and SN of MPTP-treated mice. (A) Following acute MPTP treatment, DCX immunoreactive newborn neurons (brown) are observed not only in the SVZ but also in the adjacent striatum (caudate-putamen, CPu). Scale bar=200 μ m. (B) Quantitation of DCX/BrdU-positive cells in the striatum. Data are mean $\pm$ S.E., $n=4$. * $P<0.01$ compared with saline-treated (Newman-Keuls post hoc test). (C) BrdU (red nucleus), β III-tubulin (purple cytoplasm), and DCX (green cytoplasm) co-localized within a single cell in the striatum. Scale bar=5 μ m. (D) BrdU (red nucleus), TUC-4 (purple cytoplasm), and DCX (green cytoplasm) also co-localized within a single cell in the striatum. Scale bar=5 μ m. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

Fig. 4.

FGF-2 stimulates neurogenesis in the striatum and SN of MPTP-treated mice. Quantitation of BrdU-positive profiles in SVZ (A), DCX/BrdU-positive cells in SN (B), TUC-4/BrdU-positive cells in striatum (C), β III-tubulin/BrdU-positive cells in striatum (D), and PSA-NCAM/BrdU-positive cells in SN (E). White bars, saline; black bars, MPTP. Data are mean $\pm$ S.E., $n=4$. * $P<0.01$ compared with vehicle plus saline group; # $P<0.05$, compared with vehicle plus MPTP group (Newman-Keuls post hoc test).