

Intrastriatal Transforming Growth Factor α Delivery to a Model of Parkinson's Disease Induces Proliferation and Migration of Endogenous Adult Neural Progenitor Cells without Differentiation into Dopaminergic Neurons

Oliver Cooper¹ and Ole Isacson^{1,2}

¹Harvard University and McLean Hospital, National Institute of Neurological Disorders and Stroke Udall Parkinson's Disease Research Center of Excellence, Belmont, Massachusetts 02478, and ²Department of Neurology and Program of Neuroscience, Harvard Center for Neurodegeneration and Repair, Harvard Medical School, Boston, Massachusetts 02115

We examined the cell proliferative, neurogenic, and behavioral effects of transforming growth factor α (TGF α) in a 6-OHDA Parkinson's disease model when compared with naive rats. Intrastriatal TGF α infusion induced significant proliferation, hyperplastic nodules, and substantial migratory waves of nestin-positive progenitor cells from the adult subventricular zone (SVZ) of dopamine-denervated rats. Interestingly, SVZ cells in naive rats displayed proliferation but minimal migration in response to the TGF α infusion. The cells in the expanded SVZ accumulated cytoplasmic β -catenin, indicating activation of classical Wnt signaling. However, no evidence of any neuronal differentiation was found of these recruited progenitor cells anywhere examined in the brain. Consequently, no evidence of dopaminergic (DA) neurogenesis was found in the striatum or substantia nigra in any experimental group, and amphetamine-induced behavioral rotations did not improve. In summary, the cells in the TGF α -induced migratory cellular wave remain undifferentiated and do not differentiate into midbrain-like DA neurons.

Key words: TGF α ; dopaminergic; neural progenitor; Parkinson's disease; subventricular zone; infusion

Introduction

Recent studies of adult neurogenesis have raised the awareness of potential intrinsic mechanisms to regenerate lost neurons caused by trauma or neurodegenerative disease. Remarkably, one study (Fallon et al., 2000) claimed that intrastriatal infusion of transforming growth factor α (TGF α) enables endogenous adult stem cells to generate dopaminergic (DA) neurons in the striatum of a DA denervation rat model of Parkinson's disease (PD). These morphological findings were associated with a reduced behavioral response to the dopamine agonist apomorphine, potentially reflecting reduced dopamine receptor supersensitivity (Fallon et al., 2000). TGF α is a member of the epidermal growth factor (EGF) family of proteins and is a ligand for the EGF receptor (EGFR) family of tyrosine kinase receptors (Todaro et al., 1980). The expression of TGF α is temporally and spatially coordinated with EGFR expression, which is detected in neurogenic regions such as the adult subventricular zone (SVZ) and the prenatal ventral midbrain (Seroogy et al., 1995; Kornblum et al., 1997; Doetsch et al., 2002). TGF α null mice have fewer DA neurons in

the substantia nigra pars compacta (SNc) (A9) region of the ventral midbrain prenatally, indicating a possible mitogenic effect of TGF α on the cells derived from the neuroectoderm (Blum, 1998). Adult neurogenesis of DA neurons has also recently been claimed in the substantia nigra in a study of 6-hydroxydopamine-denervated and naive rats (Zhao et al., 2003). Both Fallon et al. (2000) and Zhao et al. (2003) propose that their findings could be relevant to cell therapy for PD based on endogenous cell proliferation. The multipotent stem cell within the SVZ expresses glial fibrillary acidic protein (GFAP), a typical marker of an astrocyte (Doetsch et al., 1999a,b; Laywell et al., 2000). The transit amplifying progenitor (TAP) cells generated from these SVZ astrocytes respond to EGF, which induces their dedifferentiation into a GFAP-expressing multipotent stem cell (Doetsch et al., 2002). In turn, TAP cells generate neuroblasts that form rostrally migrating chains within the SVZ surrounded by astrocytes and TAP cells (Wichterle et al., 1997). In rodents, but apparently not patients (Sanai et al., 2004), these migrating chains converge at the anterior tip of the SVZ, forming the rostral migratory stream. The regulation of neurogenesis within the SVZ has been linked to several conditions and molecules. For example, cell-to-cell contact mechanisms via ephrin–Eph interactions regulate SVZ neurogenesis and neuroblast chain migration, whereas TGF α null mice exhibit decreased proliferation within the SVZ (Tropepe et al., 1997; Conover et al., 2000). *In vitro* experiments have shown that SVZ-derived progenitor cells can be expanded by EGF and

Received June 15, 2004; revised Aug. 25, 2004; accepted Aug. 27, 2004.

This work was supported by the Harvard Center for Neurodegeneration and Repair.

Correspondence should be addressed to Dr. Ole Isacson, McLean Hospital—Harvard Medical School, Neuroregeneration Laboratories, Mailman Research Center, 115 Mill Street, Belmont, MA 02478. E-mail: isacson@hms.harvard.edu.

DOI:10.1523/JNEUROSCI.2344-04.2004

Copyright © 2004 Society for Neuroscience 0270-6474/04/248924-08\$15.00/0

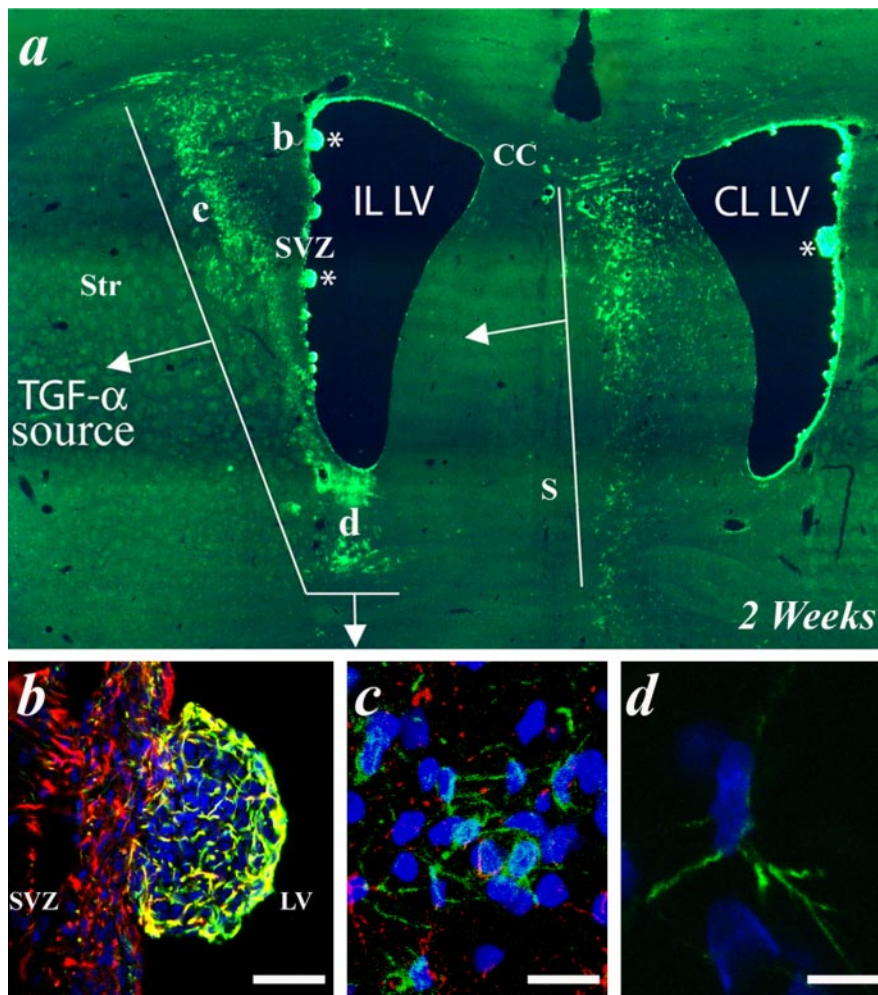


Figure 1. Representative images from 6-OHDA-lesioned rats treated with TGF α for 2 weeks. Under low magnification, two distinct nestin+ (green) waves of cells were visible (*a*). Whereas one wave appears to arise from the ipsilateral (IL) SVZ and migrates through the IL striatum toward the TGF α infusion site, the other wave appears to arise from the contralateral (CL) SVZ and migrates through the septum toward the TGF α infusion site. Within the lateral ventricle SVZ of the TGF α -treated rats, ubiquitous hypercellular nodules were present that contained both GFAP+ (red) and nestin+/GFAP+ colocalized (yellow) cells (*b*). Under higher magnification, the striatal wave of cells is composed of nestin+ cells (*c, d*). The nestin+ cells exhibit a high nuclear-to-cytoplasmic ratio and possess numerous fine processes that extend considerable distances (*d*). *, Hypercellular nodule; CC, corpus callosum; LV, lateral ventricle; S, septum; Str, striatum. Scale bars: *b*, 50 μ m; *c*, 20 μ m; *d*, 10 μ m.

fibroblast growth factor (FGF) administration (Reynolds and Weiss, 1992). Similarly, the *in vivo* administration of EGF, FGF2, and TGF α via intraventricular cannulas from osmotic pumps has altered neurogenesis in the adult brain (Craig et al., 1996; Kuhn et al., 1997; Alonso et al., 1999; Wagner et al., 1999; Fallon et al., 2000; Decker et al., 2002). In the present experiments, we examine the potential for DA neurogenesis after TGF α infusion.

Materials and Methods

Naive ($n = 8$) and adult male Sprague Dawley rats with medial forebrain bundle-administered 6-OHDA lesions previously tested by amphetamine rotation (Bjorklund et al., 2002) ($n = 17$) were obtained from Taconic Farms (Germantown, NY). The animals were maintained in accordance with current National Institutes of Health guidelines and McLean Hospital–Harvard University Institutional Animal Care and Use Committee protocols.

TGF α infusions. Rats received infusions for 1, 2, or 4 weeks. The infusions of TGF α were obtained through an intrastriatal cannula (Brain Infusion Kit II; Alzet, Cupertino, CA) (+1.2 anteroposterior; +2.7 mediolateral) from a surgically inserted interscapular osmotic pump (Osmotic Pump 2004; Alzet). The osmotic pumps delivered either 100 μ g of

TGF α (Stem Cell Pharmaceuticals, Seattle, WA) in 200 μ l of 0.1 M PBS or only PBS vehicle, both at an infusion rate of 0.25 μ l/hr. These parameters were identical to those used by Fallon et al. (2000) (J. Fallon, personal communication).

Behavioral testing. Unilaterally lesioned rats were tested for rotational behavior in response to amphetamine (4 mg/kg, i.p.) before infusion and 4 weeks postinfusion. Animals were placed (randomized) into automated rotometer bowls, and left and right full-body turns were monitored by a computerized activity monitor system, as described previously (Bjorklund et al., 2002).

Bromodeoxyuridine administration. Rats that received intrastriatal PBS or TGF α infusions for 2 weeks were administered bromodeoxyuridine (BrdU) (Sigma, St. Louis, MO) intraperitoneally for 3 d postimplantation at a dose of 50 mg/kg body mass three times per day. Rats that received intrastriatal PBS or TGF α infusions for 1 or 4 weeks were administered BrdU intraperitoneally three times a day for the day before implantation and 3 d postimplantation (4 d total) at a dose of 50 mg/kg body weight. In addition, the rats that received PBS or TGF α infusions for 1 or 4 weeks were concomitantly administered BrdU in the drinking water for 7 d postimplantation at a dose of 225 mg/kg body weight per day with the rat drinking on average 15 ml per day. At histological evaluation, there were no discernible differences between the two BrdU administration protocols used. These BrdU administration protocols were applied for between 3 and 7 d, and the protocol used by Fallon et al. (2000) was applied for 3 d. The cumulative BrdU doses used in these experiments were lower than those used by Fallon et al. (2000). The lower BrdU dosage reduced the likelihood of falsely labeled cells, but the resulting cell fates after infusion remained identifiable (Cooper-Kuhn and Kuhn, 2002; Rakic, 2002).

Histological procedures. Animals were terminally anesthetized by an intraperitoneal injection of sodium pentobarbital (100 mg/kg) and perfused with intracardial heparin saline (0.1% heparin in 0.9% saline; 100 ml/rat) followed by paraformaldehyde (4% in PBS; 200 ml/rat). The brains were removed and postfixed for 8 hr in 4% paraformaldehyde solution. After postfixation, the brains were equilibrated in 20% sucrose in PBS, sectioned (40 μ m) on a freezing microtome, and collected in PBS, as described previously (Bjorklund et al., 2002).

Immunohistochemistry and cell counting. All immunohistochemistry was performed on randomly selected series of sections that represented 1/2th of the total brain. Routine indirect immunofluorescence was performed as described previously (Bjorklund et al., 2002) using the following primary antibodies raised against tyrosine hydroxylase (TH) (1:300; Pel-Freez Biologicals, Rogers, AK), dopamine transporter (DAT) (1:2000), polysialylated neuronal cell adhesion molecule (PSA-NCAM) (1:50), neuronal nuclei (NeuN) (1:100; all from Chemicon, Temecula, CA), β -tubulin (1:500 and 1:2000; Covance, Berkeley, CA), S100 β (1:50; Swant, Bellinzona, Switzerland), GFAP (1:500; Dako, High Wycombe, UK), nestin (1 μ g/ml), 3CB2 (1 μ g/ml), 40E-C (1 μ g/ml; all from Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA), proliferating cell nuclear antigen (PCNA) (1:200), doublecortin (1:400), and β -catenin (1:100; all from Santa Cruz Biotechnology, Santa Cruz, CA). Immunohistochemistry for PCNA required antigen retrieval. Antigen retrieval was performed by incubating the free-floating sections in

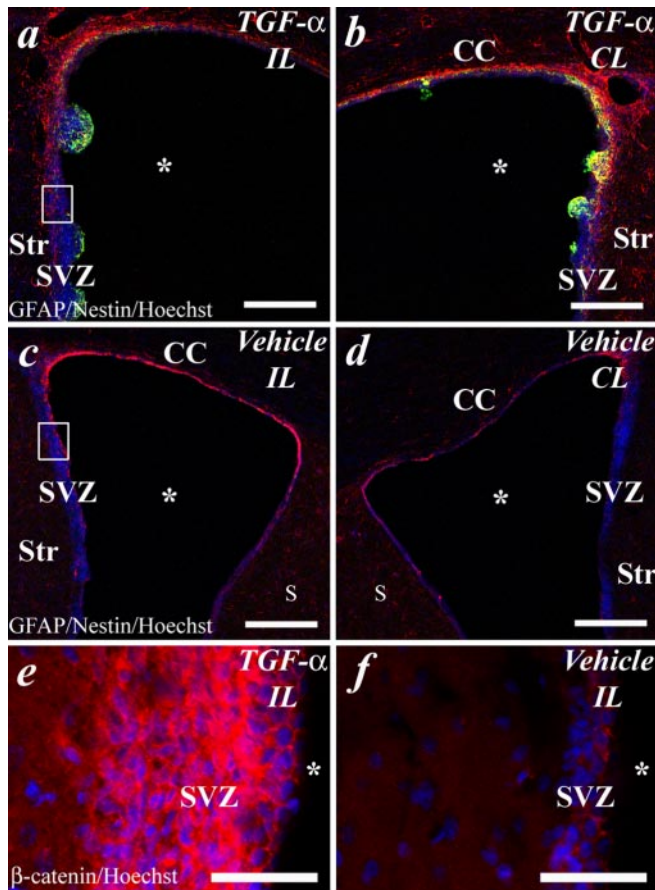


Figure 2. Representative images of SVZ in 6-OHDA-lesioned rats treated with TGF α or vehicle for 2 weeks. On examination of the bilateral SVZ at low power (*a–d*), more nestin+ (green) cells are present in the lesioned TGF α -treated SVZ (*a, b*) than in the lesioned PBS-administered SVZ (*c, d*). Within the expanded SVZ of TGF α -treated rats (*e*), β -catenin accumulated compared with PBS-administered SVZ (*f*). *, Lateral ventricle; CC, corpus callosum; S, septum; Str, striatum. Scale bars: *a–d*, 200 μ m; *e, f*, 50 μ m.

antigen retrieval solution (10 \times Target Retrieval Solution; Dako) diluted to 1 \times in distilled water. The antigen retrieval solution was preheated in a waterbath at 80°C before the sections were added for 30 min. The sections were subsequently washed three times in PBS at room temperature before immunohistochemistry was performed. Visualization of incorporated BrdU requires DNA denaturation before performing immunohistochemistry, and this was performed in a similar manner, as described previously (Kuhn et al., 1997). Sections were incubated in 2N hydrochloric acid for 30 min at 37°C before incubating in 0.1 M sodium borate for 10 min. The sections were then rinsed three times in PBS before commencing immunohistochemistry, as above. Biotinylated sheep anti-BrdU (1:200; Novus Biologicals, Littleton, CO) was diluted with other primary antibodies derived from suitable species. The BrdU was visualized using Alexa Fluor 488-conjugated streptavidin (1:500; Molecular Probes, Eugene, OR). Control sections processed without primary antibody were performed on selected sections that verified the specificity of the technique. The sections were examined using a laser scanning confocal microscope (LSM510/Meta; Carl Zeiss, Thornwood, NY). PSA-NCAM and nestin-immunopositive cell counts and SVZ measurements were performed on $\frac{1}{2}$ th of the sections using a stereology workstation (Stereoinvestigator; MicroBrightfield, Williston, VT) with an integrated epifluorescence microscope (Axioskop 2+; Carl Zeiss).

Statistical analysis. Data are presented as mean \pm SEM. ANOVA and *t* tests were used to assess differences between data groups using Statview software (SAS Institute, Cary, NC). Differences were considered statistically significant when *p* < 0.05.

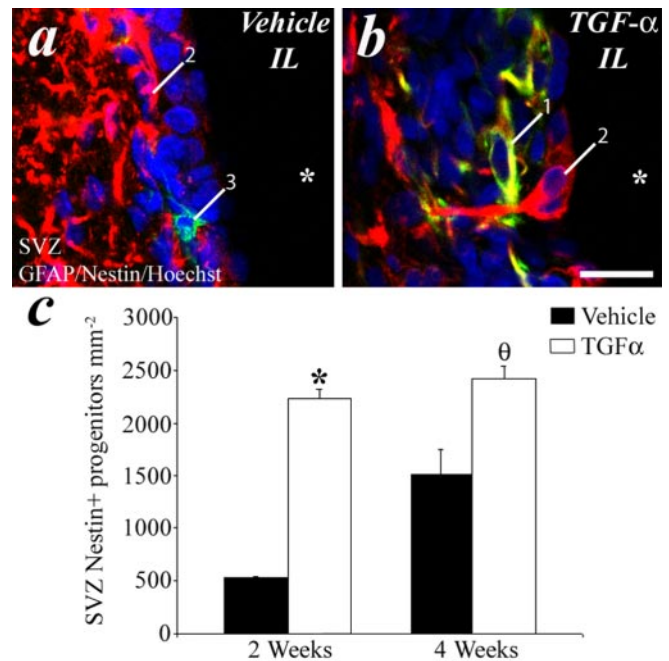


Figure 3. Intrastratial TGF α administration increases nestin+ SVZ cells. High-magnification images of the lesioned TGF α -treated SVZ (*a*) and lesioned PBS-administered SVZ (*b*) depict a thickened SVZ when TGF α was administered. The thickened SVZ contained numerous nestin+/GFAP+ (yellow) cells that exhibited an oval soma with elongated processes (1) accompanied by numerous nestin−/GFAP+ (red) cells (2). The lesioned PBS-administered SVZ is within normal limits of cellularity and contains both GFAP+ (red) cells (1) and nestin+ (green) cells (3). This difference in the number of nestin+ cells was quantified as cells per millimeter² after 2 and 4 weeks of infusion (*c*). There were significantly more nestin+ cells in the SVZ of the TGF α -treated rats (white) relative to the SVZ in the vehicle control (black) at both 2 and 4 weeks (*t* test; *p* < 0.05). *, Lateral ventricle; IL, ipsilateral. Scale bar, 20 μ m.

Results

Induction of SVZ proliferation and migration

Intrastratial infusion of TGF α into unilateral 6-OHDA-lesioned rats expanded the ipsilateral and contralateral SVZ and induced bilateral cell migratory waves rostral to the infusion site (Fig. 1*a*). Within 7 d of TGF α infusion, hyperplastic nodules formed bilaterally in the medial, lateral, and dorsal walls of the SVZ (Fig. 1*b*). Radiating in an anterior direction from these expanded SVZs, cell migratory waves formed in the adjacent striatum and septum. The cells in these migratory waves expressed nestin, a marker for multipotent progenitor cells in the developing brain (Lendahl et al., 1990), and exhibited small somata and extended fine, branched processes (Fig. 1*c, d*). The intrastratial TGF α infusion expanded a nestin+/GFAP+ cell population in the SVZ that exhibited an astrocytic morphology (Figs. 2*a, b, 3b*). The SVZ expansion and migration were not present in vehicle-administered rats (Figs. 2*c, d, 3a*). Interestingly, a general increase and change in localization of β -catenin expression was also observed in the expanded SVZ, such that the normal membranous localization was complemented by prominent cytoplasmic β -catenin (Fig. 2*e, f*). Throughout the infusion period, cells in the SVZ of vehicle-administered rats expressed the PCNA (Fig. 4*a–d*). Numerous cells in the SVZ of TGF α -treated rats also expressed PCNA (Fig. 4*e–h*). The contralateral SVZ-derived nodules continued to increase in size (>100 μ m in diameter) throughout the 4 week infusion period (Fig. 4*h*) and occasionally bridged the lateral ventricle. After 4 weeks, the ipsilateral SVZ-derived nodules were shallower and less circumscribed, and an increase in the size of the adjacent parenchymal wave of cells was observed (Fig. 4*g*).

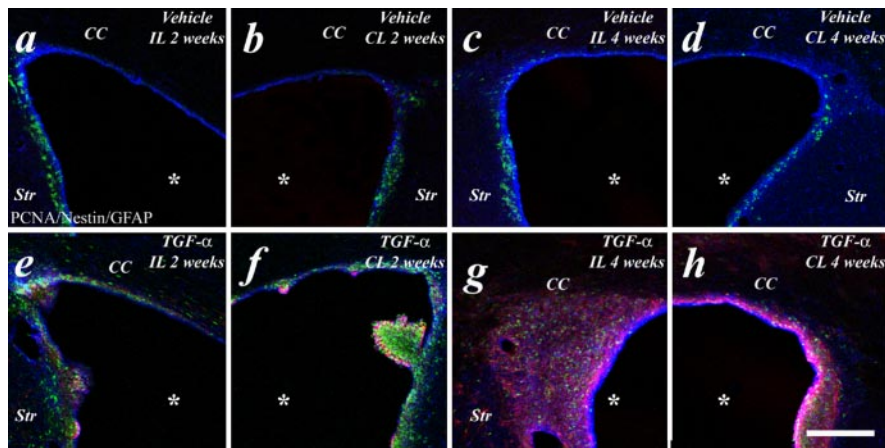


Figure 4. Representative images of SVZ in 6-OHDA-lesioned rats treated with TGF α or vehicle for 2 and 4 weeks. Vehicle-administered rats exhibited scattered nestin+ (red) cells among numerous GFAP+ (blue) cells in the SVZ with an expected number of PCNA+ (green) nuclei homogeneously distributed throughout the lateral wall (*a–d*). The ipsilateral (IL) and contralateral (CL) SVZ in vehicle-administered rats exhibited similar features at both 2 (*a, b*) and 4 (*c, d*) weeks of infusion. After 2 weeks of TGF α infusion, both ipsilateral (*e*) and contralateral SVZ (*f*) contained numerous nestin+ (red) cells with PCNA+ (green) nuclei. The hypercellular nodules present in both SVZ were composed entirely of PCNA+ nuclei. After 4 weeks of TGF α infusion, the ipsilateral SVZ (*g*) contained numerous nestin+ cells that were also present in the adjacent striatum. The ipsilateral hypercellular nodules appeared to become confluent, forming a thickened SVZ (*g*). The contralateral SVZ (*h*) was also thickened but lacked any nestin+ cells in the adjacent striatum. The hypercellular nodules in the contralateral SVZ remained prominent (*h*). *, Lateral ventricle; CC, corpus callosum; Str, striatum. Scale bar, 250 μ m.

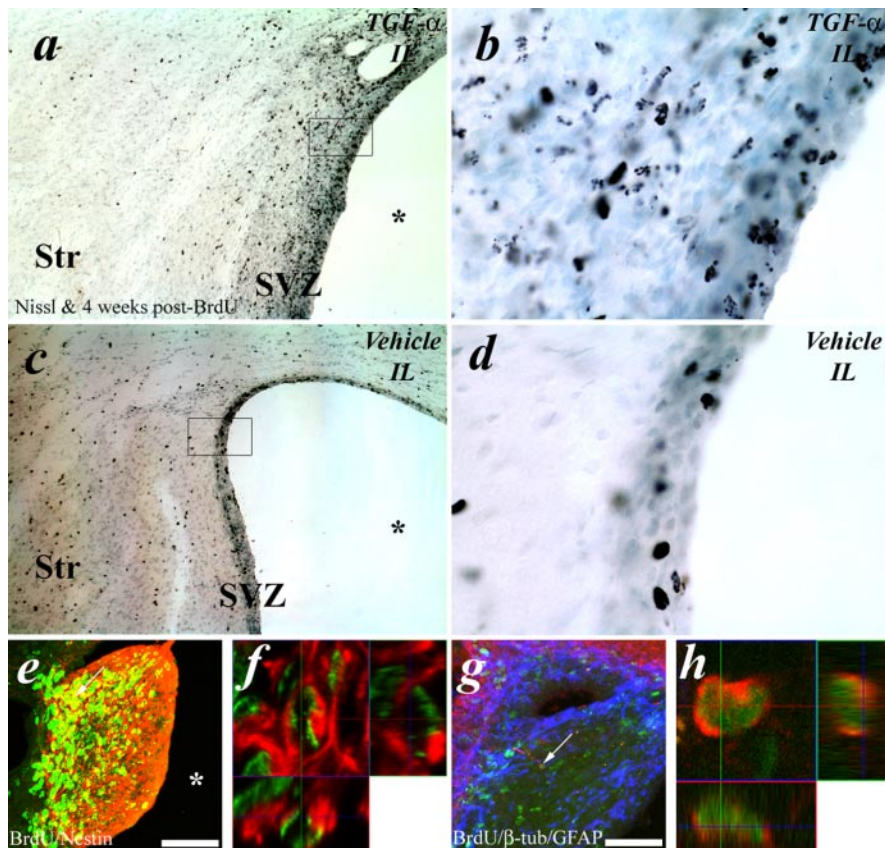


Figure 5. Representative images of BrdU-labeled cells in 6-OHDA-lesioned rats after 2 weeks infusion of TGF α or vehicle. Intrastriatal infusion of TGF α produced numerous BrdU+ cells in the ipsilateral SVZ (*a, b*) compared with vehicle-administered rats (*c, d*). The hypercellular SVZ nodules (*e*) that extend into the lateral ventricle (*) contain numerous nestin+ cells (red), but only a fraction of these cells are also BrdU+ (green). The majority of the intensely stained BrdU+ nuclei reside at the SVZ–nodule interface. At higher magnification (*f*), a proportion of the nestin+ cells (red) are BrdU+ (green). GFAP (blue) and β -tubulin (red) were also examined in the SVZ (*g*). Isolated GFAP+/BrdU+ and β -tubulin+/BrdU+ cells were present. At higher magnification (*h*), the isolated β -tubulin+ (red)/BrdU+ (green) cell was confirmed by three-dimensional confocal imaging. Str, Striatum. Scale bar, 50 μ m.

Immunohistochemical analysis demonstrated an increase in the number of BrdU+ cells in the SVZ of TGF α -treated rats (Fig. 5*a–d*). The vast majority of BrdU+ SVZ cells expressed nestin (Fig. 5*e, f*), whereas very few BrdU+ SVZ cells expressed solely β -tubulin or GFAP (Fig. 5*g, h*). Interestingly, the majority of the BrdU-positive cells within the hyperplastic nodules resided at the SVZ–nodule interface (Fig. 5*e*).

The cell types that developed in the SVZ of TGF α -administered rats differed from vehicle-administered rats. A 128% increase in the numbers of SVZ cells expressing nestin was observed after TGF α administration (Fig. 3). A concomitant 53% depletion of the PSA-NCAM-expressing cell population was observed in the SVZ (Fig. 6), indicating a loss of neuroblasts. Doublecortin is a similar marker of migrating neuroblasts (Brown et al., 2003), and the number of doublecortin-expressing cells in the SVZ was similarly diminished after TGF α infusion, corroborating the neuroblast loss characterized by PSA-NCAM (data not shown). Nestin-expressing cells and PSA-NCAM-expressing cells in the SVZ of naive animals administered TGF α or vehicle infusions were also quantified (data not shown). Although there appeared to be a difference in SVZ cell populations between naive and 6-OHDA-lesioned rats, detailed quantification did not establish a significant difference between the groups ($p > 0.05$). As expected with increased EGF agonist availability, immunohistochemistry demonstrated a marked increase of EGFR expression in the SVZ of TGF α -treated rats compared with vehicle-administered rats after 28 d of infusion (data not shown).

Within the parenchyma, ipsilateral and contralateral cell migratory waves radiated from the SVZ (Fig. 7). The cell migratory waves only appeared anterior to the TGF α infusion site (Fig. 7*a, b*). The waves of cells coexpressed nestin and PCNA in the striatum (Fig. 7*c, f*), around the anterior commissure (Fig. 7*d*), and in the septum (Fig. 7*e, g, h*), indicating that these cells were still dividing in response to the TGF α -induced mitogenic effect, but very few of the cells in the migratory waves were BrdU immunopositive. At higher magnification, the cells in the migratory waves frequently formed chains (Fig. 7*g, h*). A gradient of nestin+ progenitors was observed with the majority of cells closest to the infusion site at 2 weeks. After 4 weeks, numerous nestin+ progenitors were observed in the rostral areas of the cell migratory waves (Fig. 7*i, j*). In addition, a large number of nestin+ progenitors was observed in the contralateral cell migratory

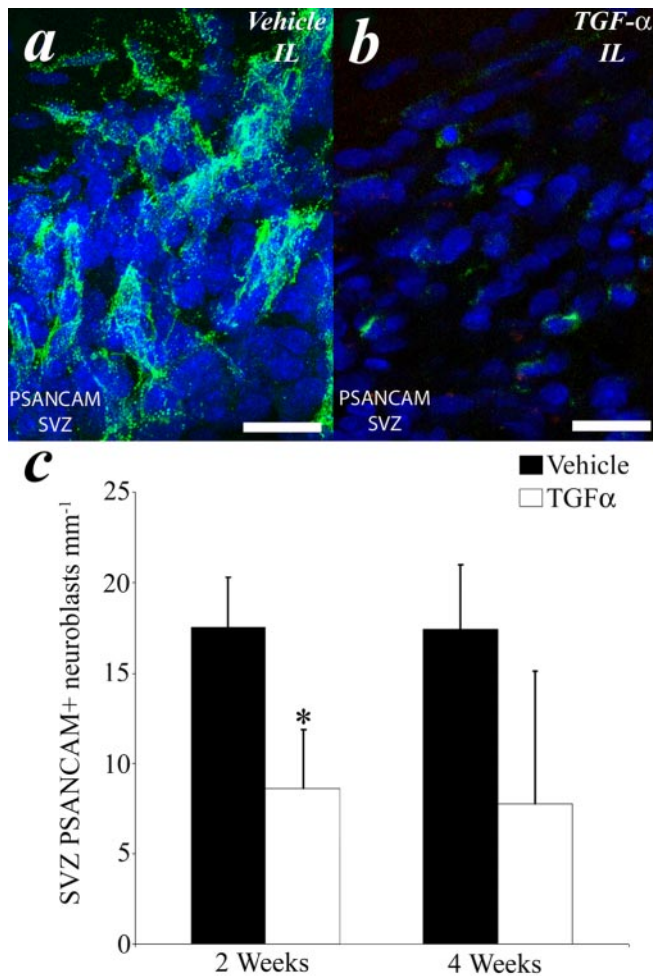


Figure 6. Intrastratial TGF α administration reduces PSANCAM+ SVZ cells. Rats that received an intrastratial infusion of vehicle demonstrated numerous PSANCAM+ cells in the SVZ (*a*), whereas rats treated with TGF α possessed relatively few PSANCAM+ cells in the SVZ (*b*). This difference was quantified as cells per millimeter after 2 and 4 weeks of infusion (*c*). There were significantly fewer PSANCAM+ cells in the SVZ of the TGF α -treated rats (white) relative to the SVZ in the vehicle control (black) (*t* test; *p* < 0.05). IL, Ipsilateral. Scale bar, 20 μ m.

wave that extended into the medial orbital cortex, prelimbic cortex, and medial olfactory bulb. In the ipsilateral hemisphere, the cell migratory wave focused into the rostral migratory stream and expanded the olfactory part of the lateral ventricle into the medial part of the anterior olfactory nucleus.

Differentiation of nestin+ progenitors

Cells expressing PSA-NCAM, doublecortin, NeuN, β -tubulin, or GFAP were not present in the striatal or septal migratory wave regions throughout the 4 week TGF α infusion period. We also immunostained postmortem tissue for TH, the rate-limiting enzyme of dopamine synthesis (Fig. 8*a*). The majority of the striata from 6-OHDA-lesioned rats contained no TH-positive neurons. This was also true for brains analyzed for non-6-OHDA-lesioned animals. Only 3 of 16 and 1 of 3 rats in the 4 week 6-OHDA lesion group contained any TH-positive cells, and one rat received a TGF α infusion. A mean of 2079 cells that had incorporated BrdU was examined for TH and DAT colocalization in the ipsilateral striatum of each rat (*n* = 4). None of these striatal TH-positive neurons were DAT- or BrdU-positive. In the SNc, a few remaining DA neurons were present in the 6-OHDA-lesioned side, but neither these neurons nor the TH-positive neurons in the con-

tralateral SNc had incorporated BrdU (Fig. 8*a*). To test whether intrastratial TGF α administration can restore motor function to rats with a unilateral 6-OHDA lesion, amphetamine rotations were used before infusion and after 4 weeks of infusion (Moore et al., 2001). No significant reductions in rotation scores were recorded using this behavioral test (Fig. 8*b*).

Discussion

In these experiments, we found a cellular proliferation in the SVZ in response to the TGF α infusion, with hyperplastic ventricular nodules and migration of neural progenitors into the adjoining striatal, septal, and cortical parenchyma in 6-OHDA-lesioned animals but not naive animals. However, there was no evidence in any of the locations examined that the mitotically active migratory cells differentiated into a mature neuronal or glial cell type. DA neurons with incorporated BrdU label were not present in the lesioned striatum or substantia nigra. This lack of DA neurogenesis in the lesioned striatum and substantia nigra was corroborated by an absence of functional recovery from the amphetamine-induced side bias in treated and control rats. Interestingly, an independent study published recently is consistent with our conclusion that there is no evidence of new dopaminergic neurons formed in the adult mammalian substantia nigra under normal conditions (Frielingsdorf et al., 2004).

In normal animals that did not have a previous dopamine denervation of the striatum, infusion of TGF α was insufficient to induce a migratory wave of nestin-expressing cells from the SVZ. However, in 6-OHDA-lesioned animals, we observed migratory waves of nestin-positive cells orientated from the SVZs of both hemispheres toward the infusion site. Although EGFR mediates chemotactic migration in the developing telencephalon, the complete set of molecular migratory cues in the dopamine-denervated striatum are unknown (Caric et al., 2001). One hypothesis is that dopamine receptors (D_1) on the nestin-positive cells are involved in limiting the proliferative response (Ohtani et al., 2003); thus, DA denervation would release these cells from such inhibition. The nestin-positive cells in the cell migratory wave also expressed PCNA, indicating that the cells were a mitotically active progenitor cell type, which exhibited a fusiform morphology consistent with the process of migration. The absence of BrdU label in the nestin-positive waves of cells may reflect a dilution of BrdU retention with successive mitoses (Dayer et al., 2003). Nonetheless, there were no neuroblasts that expressed doublecortin or PSA-NCAM in the area of the migratory progenitor cell waves. This provided evidence, independent of BrdU label, that the cells within the migratory wave were not differentiating toward a neuronal phenotype. Immunohistochemistry using 3CB2 or 40E-C antibodies showed that the wave of cells did not have a scaffold or the presence of radial glia that provide mechanisms for migration in the neocortex and telencephalon (Alvarez-Buylla et al., 2001; Rakic, 2003). In addition, these nestin+/PCNA+ cells did not express mature cellular proteins, such as NeuN, β -tubulin, or GFAP. These data points suggest that all of the cells in the wave are immature and relatively undifferentiated. Whereas the ipsilateral migratory waves of cells were solely anterior of the infusion site, the contralateral migratory waves were orientated in an anterior but more lateral direction toward the ipsilateral hemisphere. This reproducible distribution of nestin+/PCNA+ cells in the parenchyma indicates a potential gradient of stimulus associated with striatal TGF α infusion. The TGF α stimulus may cause elevated expression of developmental guidance cues in the adult brain that are normally present in the developing brain (Seroogy et al., 1995; Kornblum et al., 1997).

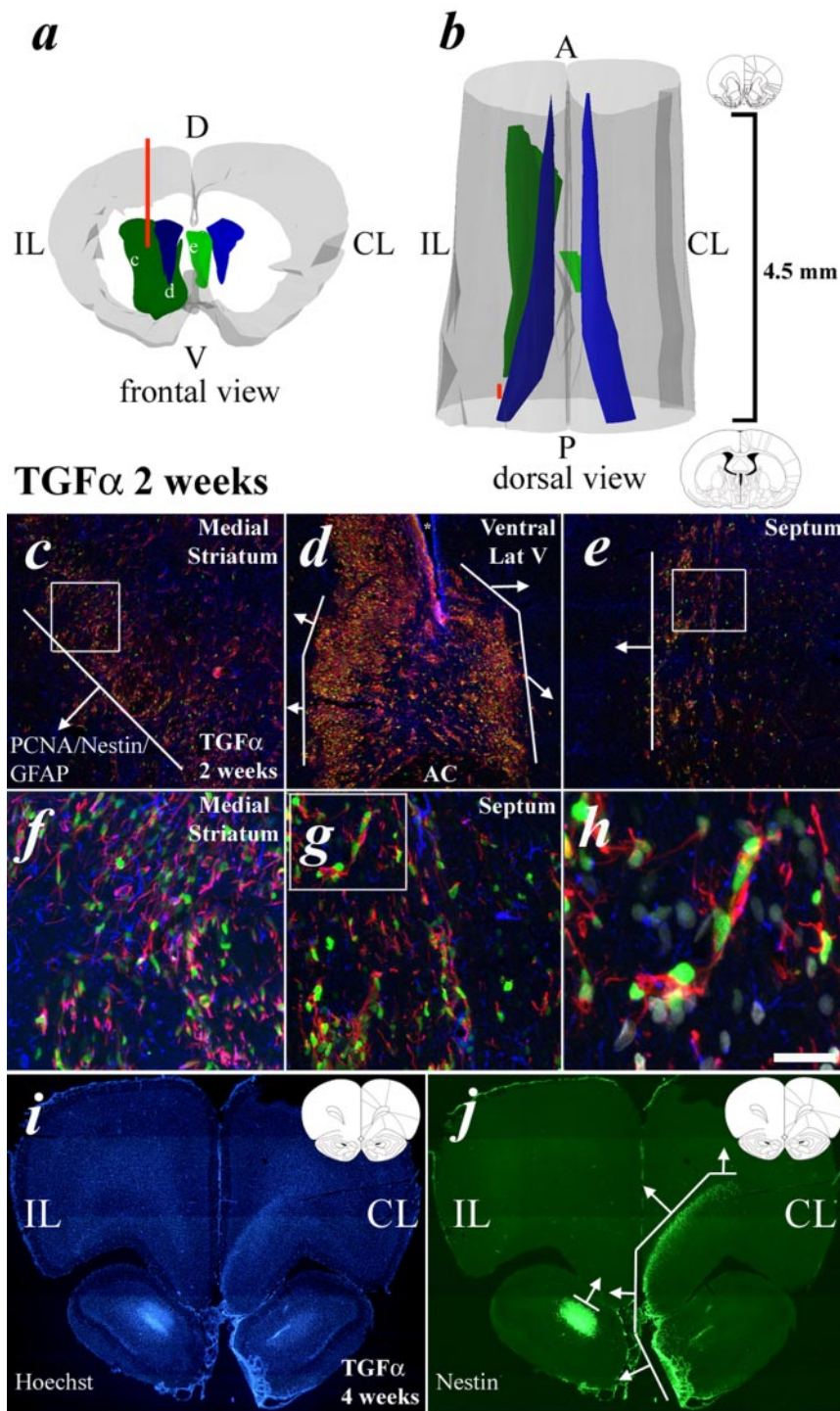


Figure 7. Characterization of the cell migratory waves. In the parenchyma (gray), ipsilateral (IL; dark green) and contralateral (CL; light green) cell, migratory waves radiated away from the respective SVZs (dark and light blue) during intrastratial infusion of TGF α (orange) (*a*). After 2 weeks, the cell migratory waves were only present anterior (A) to the infusion site, and cell densities reduced at the anterior tip of the waves (*b*). The cell migratory waves were predominantly composed of nestin+ /PCNA+ cells exhibiting a fusiform morphology (*c–h*). After 4 weeks of TGF α infusion, dopamine-denervated rats showed prominent nestin+ cellular waves in the contralateral prelimbic and orbital cortex and olfactory bulb with a hypercellular thickening of the ipsilateral olfactory ventricle lining (*i, j*). D, Dorsal; V, ventral; P, posterior; AC, antero-caudal. Scale bar, 25 μ m.

Furthermore, the effect of TGF α in the SVZ was to induce differentiation of progenitor cells, as shown by the significant depletion of SVZ neuroblasts during TGF α infusion that is consistent with an EGF infusion study (Doetsch et al., 2002). This TGF α -induced dedifferentiation may create a cell population

that is more sensitive to exogenous guidance cues (Doetsch et al., 2002). The combination of the characteristic changes in migration patterns according to the site of the infusion cannula and the consistent cell orientation indicates that the PCNA+/nestin+ cells in the parenchyma are derived from the SVZ, although non-neural cells can also express nestin in the CNS (Lardon et al., 2002).

The hypercellular SVZ-derived nodules protruding into the neighboring lateral ventricle were the result of this proliferative response. Interestingly, similar nodules have been demonstrated during intraventricular EGF infusions (Kuhn et al., 1997). In that study, the nodules resolved after cessation of infusion. However, an inherent risk of carcinogenesis may be associated with such repeated mitotic events, and high levels of EGFR expression have been linked to aggressive forms of ependymoma (Vogelstein et al., 1988; Gilbertson et al., 2002). The bilateral mitogenic effect of the TGF α infusion indicated that the protein was bioactive at comparatively long distances from the infusion site, although the proliferative effects were restricted to the SVZ of the lateral ventricles and much more prominently ipsilateral than contralateral. The increased β -catenin immunoreactivity we observed in the SVZ during TGF α administration is an interesting finding. β -Catenin was identified by its association with E-cadherin and is an integral component of classical Wnt signaling that facilitates proliferation and differentiation via cell-to-cell contact mechanisms (e.g., cadherin and Eph–Ephrin) in a variety of tissues (McCrea et al., 1991; Nelson and Nusse, 2004). β -Catenin signals are important during developmental cell growth and fate determination in the nervous system (Zechner et al., 2003). β -Catenin-mediated signaling is only active if the protein leaves the cell membrane and cytoplasmic localization is an indicator of Wnt signaling initiation leading to nuclear accumulation and target gene transcription (Huelsenken and Birchmeier, 2001; Nelson and Nusse, 2004). Our identification of this component of the signaling pathway after TGF α stimulus suggests a role for Wnt signals in the proliferative regulation of the SVZ.

After 4 weeks, no new dopaminergic neurons were found in the lesioned striatum of TGF α - or vehicle-administered rats. This contrasts with a previous report (Fallon et al., 2000) using identical methods to our study that illustrated the presence of new DA neurons identified by tyrosine hydroxylase and dopamine transporter immunoreactivity in cells containing BrdU (Fallon et al., 2000). In addition, no new dopaminergic neurons were found in the lesioned substantia nigra pars compacta, which differs from a re-

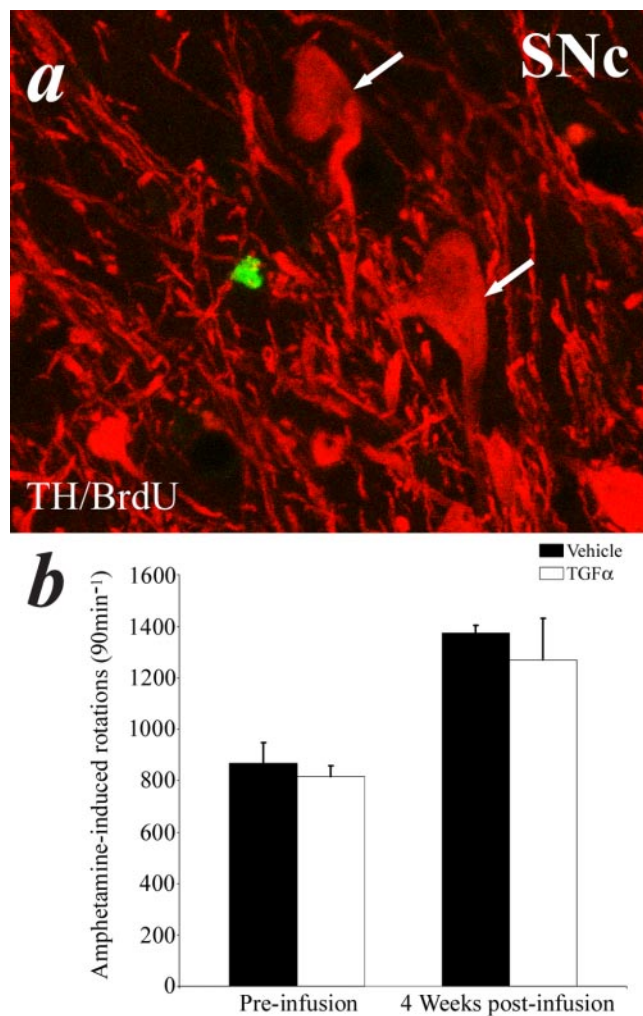


Figure 8. Lack of new DA neurons. None of the TH (red)-immunopositive neurons in the SNc of any animal in any group had nuclear incorporation of BrdU (green), although small infrequent BrdU+ nuclei were found in the region (*a*). The lack of striatal or nigral DA neurogenesis was reflected by an absence of improvement in amphetamine-induced side bias in either TGF α - or vehicle-administered rats (*b*). Scale bar, 20 μ m.

port that identified ongoing neurogenesis in that region (Zhao et al., 2003). This may be attributable to the challenges of BrdU incorporation and detection methods but still highlights the stringency required for identifying neurogenesis in novel regions of the adult brain (Kornack and Rakic, 2001; Cooper-Kuhn and Kuhn, 2002; Rakic, 2002).

Fallon et al. (2000) demonstrated a reduced apomorphine sensitivity measured by behavioral rotations after 4 weeks of intrastriatal TGF α infusion. This behavioral test relies on apomorphine stimulating supersensitive dopamine receptors in the dopamine-denervated striatum, which induces contralateral rotations in the rat. Structural perturbations in the striatum caused by cannulas or inflammation (e.g., post 6-OHDA denervation) (Dunnett et al., 1988; Jeyasingham et al., 2001) can make apomorphine behavior dopamine supersensitivity tests unreliable. The striatal structural perturbations may cause the supersensitive receptors to be lost or damaged, thus reducing apomorphine-induced side bias in a dopaminergic-independent manner (Borlongan et al., 1997). Therefore, given these well known limitations of the apomorphine test in cases of surgical perturbations of the striatum, we used amphetamine-induced side bias to investi-

gate behavioral improvement. Amphetamine acts in a similar manner to cocaine by opening the dopamine transporter, causing a sharp elevation of DA in the synaptic cleft disrupting the normal feedback control of the transmitter level (Fischer and Cho, 1979; Pierce and Kalivas, 1997). This test provides robust ipsilateral side bias, even in perturbed striata, because it more realistically reflects dopamine release capacity (Dunnett et al., 1988). The lack of motor improvement after amphetamine administration in TGF α -infused 6-OHDA-lesioned rats is consistent with the observed absence of dopamine-producing neurons within the lesioned striatum. In addition, an olfactory discrimination test showed no difference between TGF α and vehicle infusions, indicating that the TGF α infusion also does not affect behavioral tests of olfactory function via altered neurogenesis (O. Cooper, unpublished observations).

Although we reproduced the findings of proliferation and migration of neural precursors, our study contrasts with a previous report of intrastriatal TGF α infusion leading to DA neurogenesis in the adult brain because there was an absence of differentiation of such neural precursors (Fallon et al., 2000). The amount of TGF α that we administered, at least 2 weeks postlesion, was shown by Fallon et al. to be sufficient to induce a migratory ridge and improve apomorphine-induced side bias after 2 weeks of infusion (Fallon et al., 2000). Furthermore, the development of the non-neurogenic hyperplastic nodules in the SVZ clearly demonstrates effective delivery of active TGF α . The lack of neural differentiation and DA neurogenesis was congruent with our negative behavioral data that realistically reflect the dopamine release capacity of the striatum.

In the SVZ, control of the neurogenic niche is created by the expression of the bone morphogenetic protein 4 (BMP4) and the BMP antagonist noggin by SVZ astrocytes and ependymal cells, respectively (Lim et al., 2000). These two proteins interact and control neuronal–glial fate determination within the SVZ, and manipulation of this interaction by exogenous noggin enhances neuronal fate determination (Lim et al., 2000; Chmielnicki et al., 2004). Interestingly, after DA denervation, expression of BMP, BMP receptor, follistatin, cerberus, and chordin mRNA decreases in the striatum and substantia nigra, indicating altered homeostasis in these non-neurogenic zones (Chen et al., 2003). Delivery of noggin protein to the denervated striatum may create a neurogenic niche of the SVZ and promote neuronal differentiation of the migrating nestin progenitors induced by intrastriatal TGF α infusion. During development, TGF α does control the number of midbrain DA neurons, because TGF α mutations reduce midbrain DA neurons (A9 group) in newborn mice (Blum, 1998). Presumably, this action of TGF α occurs within the neuroectoderm before the cells become postmitotic and migrate ventrally and laterally. Although intrastriatal TGF α infusion provides an external mechanism to induce proliferation of cells within the adult SVZ, perhaps reconstituting neuroectoderm-like qualities, our data indicate that neither TGF α administration nor a lesioned striatum will provide sufficient cues to affect the differentiation of nestin precursors into functional DA neurons (Cameron et al., 1998). Future directions for this work will focus on providing additional signals to the SVZ-derived cells in the dopamine-denervated striatum to differentiate into functional midbrain-like DA neurons.

References

- Alonso G, Prieto M, Chauvet N (1999) Tangential migration of young neurons arising from the subventricular zone of adult rats is impaired by surgical lesions passing through their natural migratory pathway. *J Comp Neurol* 405:508–528.

- Alvarez-Buylla A, Garcia-Verdugo JM, Tramontin AD (2001) A unified hypothesis on the lineage of neural stem cells. *Nat Rev Neurosci* 2:287–293.
- Bjorklund LM, Sanchez-Pernaute R, Chung S, Andersson T, Chen IY, McNamara KS, Brownell AL, Jenkins BG, Wahlestedt C, Kim KS, Isacson O (2002) Embryonic stem cells develop into functional dopaminergic neurons after transplantation into a Parkinson rat model. *Proc Natl Acad Sci USA* 99:2344–2349.
- Blum M (1998) A null mutation in TGF- α leads to a reduction in midbrain dopaminergic neurons in the substantia nigra. *Nat Neurosci* 1:374–377.
- Borlongan CV, Cameron DF, Saporta S, Sanberg PR (1997) Intracerebral transplantation of testis-derived sertoli cells promotes functional recovery in female rats with 6-hydroxydopamine-induced hemiparkinsonism. *Exp Neurol* 148:388–392.
- Brown JP, Couillard-Despres S, Cooper-Kuhn CM, Winkler J, Aigner L, Kuhn HG (2003) Transient expression of doublecortin during adult neurogenesis. *J Comp Neurol* 467:1–10.
- Cameron HA, Hazel TG, McKay RD (1998) Regulation of neurogenesis by growth factors and neurotransmitters. *J Neurobiol* 36:287–306.
- Caric D, Raphael H, Viti J, Feathers A, Wancio D, Lillien L (2001) EGFRs mediate chemotactic migration in the developing telencephalon. *Development* 128:4203–4216.
- Chen HL, Lein PJ, Wang JY, Gash D, Hoffer BJ, Chiang YH (2003) Expression of bone morphogenetic proteins in the brain during normal aging and in 6-hydroxydopamine-lesioned animals. *Brain Res* 994:81–90.
- Chmielnicki E, Benraiss A, Economides AN, Goldman SA (2004) Adenovirally expressed noggin and brain-derived neurotrophic factor cooperate to induce new medium spiny neurons from resident progenitor cells in the adult striatal ventricular zone. *J Neurosci* 24:2133–2142.
- Conover JC, Doetsch F, Garcia-Verdugo JM, Gale NW, Yancopoulos GD, Alvarez-Buylla A (2000) Disruption of Eph/ephrin signaling affects migration and proliferation in the adult subventricular zone. *Nat Neurosci* 3:1091–1097.
- Cooper-Kuhn CM, Kuhn HG (2002) Is it all DNA repair? Methodological considerations for detecting neurogenesis in the adult brain. *Brain Res Dev Brain Res* 134:13–21.
- Craig CG, Tropepe V, Morshead CM, Reynolds BA, Weiss S, van der Kooy D (1996) *In vivo* growth factor expansion of endogenous subependymal neural precursor cell populations in the adult mouse brain. *J Neurosci* 16:2649–2658.
- Dayer AG, Ford AA, Cleaver KM, Yassae M, Cameron HA (2003) Short-term and long-term survival of new neurons in the rat dentate gyrus. *J Comp Neurol* 460:563–572.
- Decker L, Picard-Riera N, Lachapelle F, Baron-Van Evercooren A (2002) Growth factor treatment promotes mobilization of young but not aged adult subventricular zone precursors in response to demyelination. *J Neurosci Res* 69:763–771.
- Doetsch F, Caille I, Lim DA, Garcia-Verdugo JM, Alvarez-Buylla A (1999a) Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell* 97:703–716.
- Doetsch F, Garcia-Verdugo JM, Alvarez-Buylla A (1999b) Regeneration of a germinal layer in the adult mammalian brain. *Proc Natl Acad Sci USA* 96:11619–11624.
- Doetsch F, Petreanu L, Caille I, Garcia-Verdugo JM, Alvarez-Buylla A (2002) EGF converts transit-amplifying neurogenic precursors in the adult brain into multipotent stem cells. *Neuron* 36:1021–1034.
- Dunnett SB, Hernandez TD, Summerfield A, Jones GH, Arbuthnot G (1988) Graft-derived recovery from 6-OHDA lesions: specificity of ventral mesencephalic graft tissues. *Exp Brain Res* 71:411–424.
- Fallon J, Reid S, Kinyamu R, Opole I, Opole R, Baratta J, Korc M, Endo TL, Duong A, Nguyen G, Karkehabadi M, Twardzik D, Patel S, Loughlin S (2000) *In vivo* induction of massive proliferation, directed migration, and differentiation of neural cells in the adult mammalian brain. *Proc Natl Acad Sci USA* 97:14686–14691.
- Fischer JF, Cho AK (1979) Chemical release of dopamine from striatal homogenates: evidence for an exchange diffusion model. *J Pharmacol Exp Ther* 208:203–209.
- Frielingsdorf H, Schwarz K, Brundin P, Mohapel P (2004) No evidence for new dopaminergic neurons in the adult mammalian substantia nigra. *Proc Natl Acad Sci USA* 101:10177–10182.
- Gilbertson RJ, Bentley L, Hernan R, Juntilla TT, Frank AJ, Haapasalo H, Connolly M, Wetmore C, Curran T, Elenius K, Ellison DW (2002) ERBB receptor signaling promotes ependymoma cell proliferation and represents a potential novel therapeutic target for this disease. *Clin Cancer Res* 8:3054–3064.
- Huelsken J, Birchmeier W (2001) New aspects of Wnt signaling pathways in higher vertebrates. *Curr Opin Genet Dev* 11:547–553.
- Jeyasingham RA, Baird AL, Meldrum A, Dunnett SB (2001) Differential effects of unilateral striatal and nigrostriatal lesions on grip strength, skilled paw reaching and drug-induced rotation in the rat. *Brain Res Bull* 55:541–548.
- Kornack DR, Rakic P (2001) Cell proliferation without neurogenesis in adult primate neocortex. *Science* 294:2127–2130.
- Kornblum HI, Hussain RJ, Bronstein JM, Gall CM, Lee DC, Seroogy KB (1997) Prenatal ontogeny of the epidermal growth factor receptor and its ligand, transforming growth factor α , in the rat brain. *J Comp Neurol* 380:243–261.
- Kuhn HG, Winkler J, Kempermann G, Thal LJ, Gage FH (1997) Epidermal growth factor and fibroblast growth factor-2 have different effects on neural progenitors in the adult rat brain. *J Neurosci* 17:5820–5829.
- Lardon J, Rooman I, Bouwens L (2002) Nestin expression in pancreatic stellate cells and angiogenic endothelial cells. *Histochem Cell Biol* 117:535–540.
- Laywell ED, Rakic P, Kukekov VG, Holland EC, Steindler DA (2000) Identification of a multipotent astrocytic stem cell in the immature and adult mouse brain. *Proc Natl Acad Sci USA* 97:13883–13888.
- Lendahl U, Zimmerman LB, McKay RD (1990) CNS stem cells express a new class of intermediate filament protein. *Cell* 60:585–595.
- Lim DA, Tramontin AD, Trevejo JM, Herrera DG, Garcia-Verdugo JM, Alvarez-Buylla A (2000) Noggin antagonizes BMP signaling to create a niche for adult neurogenesis. *Neuron* 28:713–726.
- McCrea PD, Turck CW, Gumbiner B (1991) A homolog of the armadillo protein in *Drosophila* (plakoglobin) associated with E-cadherin. *Science* 254:1359–1361.
- Moore AE, Cicchetti F, Hennen J, Isacson O (2001) Parkinsonian motor deficits are reflected by proportional A9/A10 dopamine neuron degeneration in the rat. *Exp Neurol* 172:363–376.
- Nelson WJ, Nusse R (2004) Convergence of Wnt, beta-catenin, and cadherin pathways. *Science* 303:1483–1487.
- Ohtani N, Goto T, Waeber C, Bhide PG (2003) Dopamine modulates cell cycle in the lateral ganglionic eminence. *J Neurosci* 23:2840–2850.
- Pierce RC, Kalivas PW (1997) Repeated cocaine modifies the mechanism by which amphetamine releases dopamine. *J Neurosci* 17:3254–3261.
- Rakic P (2002) Adult neurogenesis in mammals: an identity crisis. *J Neurosci* 22:614–618.
- Rakic P (2003) Elusive radial glial cells: historical and evolutionary perspective. *Glia* 43:19–32.
- Reynolds BA, Weiss S (1992) Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science* 255:1707–1710.
- Sanai N, Tramontin AD, Quinones-Hinojosa A, Barbaro NM, Gupta N, Kunwar S, Lawton MT, McDermott MW, Parsa AT, Verdugo JM, Berger MS, Alvarez-Buylla A (2004) Unique astrocyte ribbon in adult human brain contains neural stem cells but lacks chain migration. *Nature* 427:740–744.
- Seroogy KB, Gall CM, Lee DC, Kornblum HI (1995) Proliferative zones of postnatal rat brain express epidermal growth factor receptor mRNA. *Brain Res* 670:157–164.
- Todaro GJ, Fryling C, De Larco JE (1980) Transforming growth factors produced by certain human tumor cells: polypeptides that interact with epidermal growth factor receptors. *Proc Natl Acad Sci USA* 77:5258–5262.
- Tropepe V, Craig CG, Morshead CM, van der Kooy D (1997) Transforming growth factor- α null and senescent mice show decreased neural progenitor cell proliferation in the forebrain subependyma. *J Neurosci* 17:7850–7859.
- Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM, Bos JL (1988) Genetic alterations during colorectal-tumor development. *N Engl J Med* 319:525–532.
- Wagner JP, Black IB, DiCicco-Bloom E (1999) Stimulation of neonatal and adult brain neurogenesis by subcutaneous injection of basic fibroblast growth factor. *J Neurosci* 19:6006–6016.
- Wichterle H, Garcia-Verdugo JM, Alvarez-Buylla A (1997) Direct evidence for homotypic, glia-independent neuronal migration. *Neuron* 18:779–791.
- Zechner D, Fujita Y, Hulsken J, Muller T, Walther I, Taketo MM, Crenshaw 3rd EB, Birchmeier W, Birchmeier C (2003) beta-Catenin signals regulate cell growth and the balance between progenitor cell expansion and differentiation in the nervous system. *Dev Biol* 258:406–418.
- Zhao M, Momma S, Delfani K, Carlen M, Cassidy RM, Johansson CB, Brismar H, Shupliakov O, Frisen J, Janson AM (2003) Evidence for neurogenesis in the adult mammalian substantia nigra. *Proc Natl Acad Sci USA* 100:7925–7930.