In vivo gene delivery to proliferating cells in the striatum generated in response to a 6-hydroxydopamine lesion of the nigro-striatal dopamine pathway

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The degeneration of neurons in the mammalian brain is commonly associated with the division of cells located in the damaged area. The aim of the present study has been to characterise the phenotype of newly born cells in the striatum of adult rats following 6-hydroxydopamine lesion of the nigro-striatal pathway. Newborn cells were identified through labelling with either bromodeoxyuridine or retrovirus encoding green fluorescence protein. We report here that the overwhelming majority of cells resembling neuroblasts, however these cells did not appear to survive. Noggin transduction did not result in the generation of new neurons, but interestingly, greatly increased the number of oligodendrocytes generated from newborn cells.

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Introduction

Cell division occurs throughout the adult brain both constitutively and also in response to injury. Although it was long thought no new neurons were born in the adult brain, studies by Altman et al. in the 1960’s (Altman and Das, 1966) and later by many others have shown that in certain ‘neurogenic’ regions of the brain, such as the subventricular zone (SVZ) and the subgranular zone of the dentate gyrus, dividing cells do indeed give rise to new neurons. These findings have stimulated a good deal of research into the idea that neurons lost in the adult brain through disease or injury may be replaced from endogenous neural cells under certain circumstances. In fact, recent studies have reported that following neuronal death in structures lying adjacent to neurogenic brain regions, the newly generated neurons in the SVZ or subgranular zone may be recruited into the damaged area (Gould and Tanapat, 1997; Parent et al., 1997; Arvidsson et al., 2002; Magavi and Macklis, 2002; Nakatomi et al., 2002; Parent et al., 2002; Lie et al., 2004; as reviewed by Emsley et al., 2005). Although the newly formed neurons are few in number, their presence suggests that they could potentially represent a new source of neurons to replace those lost through injury. Less is currently known, however, about the fate of endogenous cells that divide outside neurogenic regions of the brain in response to injury or disease and whether these cells possess the ability to give rise to newborn cells in situ. This has been the topic for the present study, where we have sought to identify and characterise cells that divide in an animal model of Parkinson’s disease and to alter the fate of these cells through the introduction of genes known to be important for neurogenesis in the developing and adult brain.

Parkinson’s disease (PD) pathology is characterised by degeneration of the mesencephalic dopamine (DA) neurons that reside in the substantia nigra (SN) and send projections to the forebrain. The degeneration of these neurons in animal models of PD is associated with the proliferation of local cells both at the level of the midbrain and also in the striatal terminal field (Kay and Blum, 2000). Two recent studies have examined cell proliferation within the SN in rodent models of PD and reported that the regeneration of DA neurons either does not occur (Frielingsdorf et al., 2004) or at best is a rare event (Zhao et al., 2003). Even if it was in fact possible to generate reasonable numbers of new DA neurons from endogenous precursors in the adult SN, transplantation studies have indicated that these cells lack the ability to send axons to the striatum (Bentlage et al., 1999) where they normally modulate the activity of striatal projection neurons in order to maintain motor function. We have therefore chosen to examine and target the cells that divide in the striatal parenchyma following...
6-hydroxydopamine (6-OHDA) lesion of the nigrostriatal projection system. Previous studies aimed at characterising the identity of newborn cells in the adult brain have relied largely on use of the nuclear marker bromodeoxyuridine (BrdU) along with the expression of phenotypic proteins. Although this has been a highly useful approach, limitations both in the availability of good phenotypic markers and in the ability to co-localise such markers with BrdU have meant that it has only been possible to reveal the identity of a relatively small subset of newborn cells in these types of experiments. We have therefore chosen to complement this approach with the use of retroviral delivery of green fluorescent protein (GFP), which fills the cytoplasm of newborn cells, thus giving fine morphological detail. We report that no neurons are formed in the striatum in response to the 6-OHDA lesion and that the majority of the cells have glial characteristics based on the expression of phenotypic markers and cell morphology.

In order to manipulate the fate of dividing striatal cells in favour of neurogenesis we have made use of bi-cistronic retroviral vectors to constitutively express GFP and either neurogenin2 (Ngn2) or noggin in the newborn cells following 6-OHDA lesion. Ngn2 is a bHLH transcription factor that has been shown to play a critical role in the development and/or phenotypic specification of a variety of projection neurons in the developing nervous system, including DA neurons (Thompson et al., 2006). Noggin is a secreted protein that allows for the neuronal differentiation of progenitors within the adult SVZ through antagonism of BMP signaling (Lim et al., 2000). We report here that although cells transduced with Ngn2 had an immature neuroblast morphology and expressed certain neuronal markers soon after transduction, these cells did not appear to survive and differentiate into mature neurons. Noggin did not have any effect on the ability of newborn striatal cells to form new neurons. Interestingly, however, noggin transduction greatly increased the number of newborn oligodendrocytes in the striatum of 6-OHDA lesioned animals.

Materials and methods

Animal handling and surgical procedures

For all experiments, a total of fifty-four eight-week old adult female Sprague–Dawley rats (225–250 g) from B&K Universal, Stockholm, Sweden, was used. Animals were housed two–three to a cage, in temperature-controlled conditions under a twelve-hour light/dark cycle with food and water supplied ad libitum. All surgical procedures were performed according to the rules set by the Ethical Committee for Use of Laboratory Animals at Lund University.

For all surgical procedures animals were anaesthetised with a mixture of Hypnorm (0.315 mg fentanyl and 0.2 mg fluanisone/ml; Janssen Pharmaceutica, Sweden) and Dormicum (5 mg/ml medozolam; Roche AB) injected intraperitoneally (2.7 ml/kg). After surgical procedures animals were given subcutaneous injections (0.167 ml/kg) of Temgesic (0.3 mg/ml buprenorphinum; Schering-Plough) in order to promote recovery and also maintain analgesia.

Medial forebrain bundle lesion

A total of forty-nine animals were given medial forebrain bundle lesions. The animals were placed in stereotactic frames (Kopf Instruments; Germany) and 6-hydroxydopamine (3.0 µg/µl free base dissolved in 0.9% w/v NaCl with 0.05% w/v L-ascorbic acid) was delivered at two sites using a Hamilton (10 µl) syringe fitted with a 27.5 gauge needle (2.5 µl at 4.4 mm caudal and 1.2 mm lateral to bregma, 7.8 mm below the dura, toothbar set at +3.4 mm). The completeness of the lesion was retrospectively confirmed by immunohistochemistry showing complete removal of tyrosine-hydroxylase positive fibers in the ipsilateral striatum.

Bromodeoxyuridine delivery

Four lesioned animals received two intra-peritoneal injections of BrdU (50 mg/kg; Sigma) in 0.1 M phosphate buffered saline on each of days three and four after lesioning, at 12 hour intervals. This timeframe was chosen based on previous experiments where this has been observed to be a peak period of cell division in the striatum under these conditions (D.I and L.T. unpublished observations).

Retroviral gene delivery

An additional fifty lesioned animals received stereotaxic intracranial retroviral injections 3 days after 6-OHDA delivery, using a Hamilton (10 µl) syringe with a 27.5 gauge needle and fitted with a pulled glass capillary pippette (outer diameter 60–80 µm). A total of 2 µl of murine stem cell virus-based (Clontech) bi-cistronic retrovirus was injected into the striatum (0.5 mm anterior and 3 mm lateral to bregma, 5.0 mm below the dura, toothbar at –2.3 mm). Three different constructs were used encoding enhanced green fluorescent protein eGFP (Clontech) preceded by internal ribosomal entry sequence 2 in order to deliver either GFP alone (CMV-IRES2-eGFP); Ngn2 and eGFP (CMV-Ngn2-IRES2-eGFP, cDNA obtained from Dr. Jonas Frisén); or noggin and GFP (CMV-Noggin-IRES2-eGFP; cDNA obtained from Dr. Aris Economides). Both noggin and Ngn2 cDNA sequences were subcloned into the multiple cloning site of the bi-cistronic eGFP vector (Clontech).

Five non-lesioned control animals received retrovirus only encoding GFP. All viruses used were at a titre of approximately 1 × 10⁸ transducing units (TU)/ml.

Tissue processing

Animals were sacrificed at 3, 7, 14 and 42 days after retrovirus delivery (n = 5 for each group) or 2 days after BrdU delivery (n = 4). Animals were overdosed with 1 ml pentobarbital (60 mg/ml; Apoteket Production & Laboratorie, Stockholm, Sweden) and perfused transcardially with 50 ml 0.9% saline, followed by 200 ml 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer, pH 7.4. All solutions were kept cold. After decapitation the brains were dissected, removed from the skull, post-fixed for 2 h in 4% PFA at 4 °C and subsequently transferred to 25% sucrose for cryoprotection. Eight series of 40 µm sections were cut by a freezing slide microtome in the coronal plane and were stored at –20 °C.

Immunohistochemistry

All specimens were processed as free-floating sections for immunohistochemical staining procedures as previously described (Thompson et al., 2005). Quenching with 3% hydrogen-peroxide (H₂O₂) was performed on sections where 3′diaminobenzidine (DAB) was used as a chromagen. All incubation solutions contained 5% normal serum (NS, from the same species as the secondary antibody was raised in) and 0.25% Triton-X in 0.02 M potassium-phosphate buffer (KPBS) and were performed at room temperature (RT). Sections were rinsed three times between each incubation step. All staining procedures included pre-incubation for 1 h (in KPBS, 0.25% Triton-X and 5% serum for each antibody) followed by incubation overnight with primary antibody. The following primary
antibodies and dilution factors used in this study were as follows: chicken-anti GFP (1:5000, Chemicon); rabbit-anti NG2 (1:200, Chemicon); rabbit-anti-DARP-32 (1:1000, Chemicon); mouse-anti-NF 100 (1:200, Chemicon); rabbit-anti-GFAP (1:1000, Sigma); mouse-anti-CN Pase (1:100, Sigma); rabbit-anti GABA (1:250, Sigma); mouse-anti-calcibindin (1:1000, Sigma); mouse-anti-βIII (1:300, DAKO); mouse-anti-Hu (1:500, Molecular Probes); mouse-anti-OX-42 (1:100, Serotec); ED-1 (1:200, Serotec); ED-2 (1:200, Serotec); OX-1 (1:200, Serotec); rabbit-anti-MEIS2 (1:5000, Gift from A. Buchberg); rabbit-anti-PBX (1:400, Santa Cruz Biotech); mouse-anti APC-7 (1:200, Calbiochem); rabbit-anti PDGF-α (1:200, Santa Cruz Biotech); and goat-anti doublecortin (1:400, Santa Cruz Biotech). For detection, sections were incubated with biotinylated or fluorophore-conjugated secondary antibodies (1:200, 2 h; Jackson Laboratories), including biotinylated goat-anti-rabbit, Cy2 conjugated donkey-anti-chicken or streptavidin, and Cy3 conjugated donkey-anti-rat or streptavidin. Fluorophore labelled sections were mounted on chrome–alum-coated Plus slides and cover-slipped with PVA-DABco. For visualization of DAB, the avidin–biotin–complex method was used (2 h; ABC, Vectastain) following incubation with the secondary antibody. These sections were dehydrated and delipidated in increasing concentrations of ethanol, cleared in xylene and cover-slipped in Depex. Fluorescent immunohistochemistry was examined by confocal laser-scanning microscopy (Leica DMRE) equipped with green helium/neon, helium/neon and argon lasers.

Quantification and statistical analysis

Four 6-OHDA lesioned rats were used to quantify the number of BrdU or doublecortin (DCX) labelled cells in the ipsilateral and contralateral SVZ or periventricular striatum (within 0.5 mm of the SVZ), respectively. A conventional light microscope with a 40× objective was used for this quantification. All cells were counted bilaterally from four sections per rat spanning the striatum (located between 1.5 and 0.0 mm anterior to bregma) as represented in a 1 in 8 parallel series of 40 μm coronal sections. Average cell numbers in lesioned and non-lesioned striatal SVZ and periventricular areas were compared using ANOVA (α<0.05 for statistical significance). A confocal laser-scanning microscope (Leica DMRE) was used to assess the percentage of GFP-positive cells that co-expressed APC-7 or PDGF-α within the striatum of five animals per group injected with either RV-GFP or RV-GFP/noggin. A minimum of 50 GFP-positive cells per animal was counted for each group. Statistical comparison of APC-7 or PDGF-α expression in GFP-positive cells from either the RV-GFP or RV-noggin/GFP groups was performed using ANOVA (α<0.05 for statistical significance).

Results

Cell proliferation in the striatum 3 days after 6-OHDA delivery

In order to assess the early proliferative response in the striatal parenchyma following 6-OHDA delivery into the MFB, animals received i.p. delivery of BrdU on days 3 and 4 after the 6-OHDA lesion and were sacrificed on day 6. Immunohistochemistry revealed many BrdU positive cells widely and uniformly distributed throughout the parenchyma in the ipsilateral striatum (Figs. 1B, E, F). Many of the BrdU positive cells within the parenchyma appeared as doublets (see examples in Figs. 1E, F). The contralateral striatum contained substantially fewer BrdU positive cells (Fig. 1A) appearing similar to BrdU labelling seen in intact animals (not shown). BrdU positive cells were also found in the ipsilateral and contralateral SVZ but the number of BrdU positive cells did not differ significantly between the...
two sides, ipsilateral SVZ 172.8 $\pm$ 10.1 compared to the contralateral SVZ 169.3 $\pm$ 10.6. We also examined the levels of newly generated neuroblasts associated with the SVZ, through immunohistochemistry for the early neuronal progenitor marker, doublecortin (DCX). Scattered DCX-positive cells could be found within and adjacent to the SVZ, up to a distance of about 0.5 mm from the ventricle (Figs. 1C, D). There was no significant difference in the number of DCX-positive cells with in the periventricular zone between the ipsilateral 7.8 $\pm$ 1.1 and contralateral side 5.8 $\pm$ 1.3.

Identification of dividing cells through retroviral GFP delivery

In order to examine the fate and the phenotypic profile of the proliferating cells in the striatum, a murine stem cell virus encoding GFP was delivered to the ipsilateral striatum 3 days after the 6-OHDA lesion and also to intact animals. Animals were subsequently sacrificed 3 days and 1, 2 and 6 weeks after retrovirus delivery. At 3 days after virus injection (i.e. 6 days after 6-OHDA lesion) GFP immunohistochemistry revealed many GFP-positive cells along or near the needle track in both lesioned and intact animals (Fig. 2), extending from the striatum into the overlying cortex. Fewer cells could be identified distal to the needle track in intact animals compared to lesioned animals (Fig. 2).

At later time-points (7, 14 or 42 days after retrovirus delivery) the overall number of GFP-expressing cells did not appear to change in 6-OHDA lesioned animals, but they could be found more widely distributed in the striatal parenchyma, or associated with blood vessels, in addition to the clustering around the needle track (not shown).

The majority of the GFP-expressing cells were found to have glial-like morphologies. Although the predominant morphological phenotype at all time-points examined was that of astroglia with small cell bodies and ramified processes (Figs. 2C, D), these cells rarely co-expressed phenotypic markers for astrocytes such as GFAP or S100$\beta$ (Figs. 3A, B). Furthermore, while many ED-2 and OX-1 positive cells were seen in the 6-OHDA lesioned striatum (phenotypic markers for perivascular macrophages and circulating leukocytes, respectively) none of them were found to be GFP positive (Figs. 3C, D). Occasional GFP-positive cells, located close to the injection track, expressed NG2 and OX-42, phenotypic markers for immature glia and microglia respectively (Figs. 3E–J). No GFP-positive cells with neuronal morphology or expressing neuronal markers such as $\beta$-III tubulin, DCX, Hu, Map-2, DARPP-32 or calbindin were detected at any time-point (data not shown).

Not all of the glial-like cells had a definitive astroglial morphology. Some cells had larger cell bodies and many processes arranged in a uniform spherical pattern (e.g. Fig. 3B). Some of these cells expressed markers for oligodendrocytes including APC-7 and PDGF-$\alpha$, (Figs. 4A–F). However, none expressed CNPase (Figs. 4G–I). In animals sacrificed at 2 weeks after retroviral delivery, 27.7 $\pm$ 2.5% of the GFP-positive cells were found to co-express APC-7, and 17.2 $\pm$ 4.6% co-expressed PDGF-$\alpha$.

Fig. 2. GFP labelled cells in the non-lesioned striatum 3 days after viral GFP delivery (A) and ipsilateral striatum in a unilateral MFB 6-OHDA lesioned adult rat 3 days after viral GFP delivery (B). C,D: GFP labelled cells with astroglial morphology (C). Higher magnification of GFP labelled cell (3 days) (D). Scale bars: (A and B)=260 $\mu$m; (C)=60 $\mu$m; (D)=15 $\mu$m.
Fig. 3. Immunohistochemistry for GFP and phenotypic markers in unilateral MFB lesioned striatum 1 week after retroviral delivery. The majority of GFP-positive cells have an astroglial morphology with ramified processes. Most of the cells did not express S100β (A), GFAP (B), OX-1 (C), and ED-2 (D). However, some GFP-positive cells co-expressed NG2, a marker for glial cells (E–G) or OX-42, a marker for microglia (H–J). GFP in green; phenotypic markers in red. Many GFP-positive cells were found to be closely associated with blood vessels (D). Scale bars: (A–D)=60 μm; (G)=5 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 4. Immunohistochemical detection of GFP-positive cells co-expressing immature oligodendroglial markers 2 weeks after retroviral gene delivery — PDGF-α (A–C), APC-7 (D–F), but not the mature oligodendroglial marker, CNPase, (G–I) (GFP in green, phenotypic markers in red, overlap appears yellow). Scale bars: (A)=5 μm; (G)=60 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Retroviral gene delivery of noggin to proliferating striatal cells

We sought to determine if over-expression of the Noggin gene in dividing cells in the striatal parenchyma would promote de-novo neurogenesis. Following 6-OHDA lesion, proliferating cells in the striatum were transduced with a retrovirus encoding noggin together with GFP from the same bi-cistronic retroviral vector. Immunohistochemistry for GFP at 1 week after retroviral delivery indicated that a substantial proportion of the transduced cells had an oligodendrogial morphology. This was in clear contrast to cells that were transduced with GFP alone, where the predominant morphological phenotype was that of astroglia and microglia, with only few examples of cells with oligodendrogial like morphology. The noggin transduced cells were often found to be closely associated with myelinated fibre bundles with their processes projecting into the bundles, while their cell bodies were located either at or near the periphery of the fibre bundles (Figs. 5A, B) and in some cases were found to have their cell bodies and projections closely associated to striatal projection neurons (Fig. 5C). The majority, 64.7±10.6% of these cells were found to co-express APC-7, as compared to 27.4±5.7% in the GFP only.

![Fig. 5. Brightfield images of noggin-GFP transduced cells 1 week after retroviral gene delivery with morphological profile indicative of differentiated oligodendrocytes (A, B). Dashed lines demarcate myelinated fibre bundles. Cell bodies were often located in the periphery of fiber bundles with polarized projections towards the bundle. (B) Higher magnification of a GFP-positive cell with polarized projection within white matter fiber tract. (C–L) Immunohistochemical identification of noggin-GFP transduced cells in the ipsilateral striatum 2 weeks after MFB lesion. Some noggin-GFP over-expressing cells were found intimately associated with striatal projection neurons that express DARPP-32 (C). Noggin-GFP transduced cells expressed oligodendroglial phenotypic markers CNPase, (D–F); PDGF-α, (G–I); APC-7 (J–L). GFP in green; phenotypic markers in red. Scale bars: (A)=60 μm; (B)=15 μm; (C)=60 μm; (L)=15 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
controls. Consistent with an oligodendroglial phenotype many of the RV-noggin GFP cells also co-expressed PDGF-α and CNPase (Figs. 5D–I). No GFP-expressing cells were identified that had clear neuronal morphology or that co-expressed β-III tubulin, PBX, Meis2, DARP-32 or GABA (not shown). At 2 and 6 weeks following gene delivery, noggin-GFP-expressing cells were still present and maintained similar morphological and phenotypical profiles (not shown).

**Retroviral gene delivery of Ngn2 to proliferating striatal cells**

In a second series of experiments, we delivered Ngn2 combined with GFP in the same bi-cistronic retroviral construct, as described above. Immunohistochemistry revealed that 7 days after vector delivery the Ngn2-GFP transduced cells clearly did not have the same glial-like morphology as found in the two previous gene delivery groups (Fig. 6). Many of these cells had an unhealthy appearance with swollen cell bodies and dystrophic dendrites (e.g. Figs. 6B, F). In some rare cases, Ngn2-GFP-expressing cells had elongated branched projections and mature neuron-like morphologies (Figs. 6A, D). None of these cells were found to express neuronal markers such as NF 70, NF160, β-III tubulin, calbindin, DARPP-32 or GABA (data not shown), although in a few rare cases these cells were found to co-express neuron-specific proteins such as PBX (Figs. 6D, G–I). By the second week after virus injection, the GFP-expressing cells were fewer in number, and most had an unhealthy-looking morphology with disorganized and disrupted projections and some of these degenerating cells were found to co-express caspase-3 (Fig. 6). By the sixth week very few GFP-positive cells could be identified.

**Discussion**

Retroviral GFP labelling is a highly useful tool for characterisation of newly born cells within the parenchyma of brain tissue, allowing morphological characterisation of cells that otherwise do not express many of the currently available phenotypic markers used to identify neural cell types in vivo. This method, in conjunction with BrdU labelling, also helps to confirm that these cells are indeed proliferating cells and not merely replicating DNA as part of a reparative or apoptotic process (Rakic, 2002). Other studies have combined Ki-67 or PCNA and BrdU labelling (Jiang et al., 2002) to provide similar evidence. While PCNA and Ki-67 are useful in identifying proliferating cells, these transiently expressed proteins cannot be used in combination with phenotypic markers to identify the phenotypes of the cells once they have exited the cell cycle. Through immunohistochemical detection of GFP following RV-GFP transduction, we find that the predominating morphology of newly born cells in the striatum in response to 6-OHDA lesion is indicative of a glial phenotype, which is in agreement with previous studies reporting that BrdU positive cells generated in the striatum following MPTP treatment co-express glial proteins such as S100β or GFAP (Kay and Blum, 2000; Mao et al., 2001) and that microglial cells proliferate following treatment with 6-OHDA (Reimers et al., 2005). Furthermore, we report that it is possible to modify the
phenotype of newly born cells in the adult striatum by introducing either the noggin or neurogenin2 gene under the control of a constitutive promoter.

**BMP signalling and striatal proliferation**

Neurogenesis occurs constitutively in two discrete locations in the adult brain — the subgranular zone of the hippocampus and the SVZ lying between the striatum and the lateral ventricle (Gage, 2002). Recent work has shown that, in the SVZ, antagonism of BMP signalling by noggin is critical for allowing the adoption of neuronal fate by SVZ progenitor cells (Zimmerman et al., 1996; Lim et al., 2000) and thus noggin is thought to create a permissive environment for neurogenesis in the SVZ. Furthermore, manipulation of this signalling pathway through administration of exogenous noggin has been shown to enhance neurogenesis from SVZ progenitors (Lim et al., 2000; Chmielnicki et al., 2004). Here we chose to over-express noggin in striatal cells that divide in response to injury, in an attempt to create an environment that favoured neurogenesis upon differentiation. Notably, following a 6-OHDA lesion there is an upregulation of BMPR-1A and 1B mRNAs in the striatum, suggesting that signalling through this pathway is indeed present in this setting (Chen et al., 2003). To antagonise BMP signalling, we have used a mutated form of noggin in which the B2 heparin binding domain has been deleted. The resulting secreted noggin protein diffuses more extensively than wild-type noggin and thus, in addition to acting in an autocrine manner, may antagonise BMP signalling in surrounding cells (Paine-Saunders et al., 2002). Our results show that over-expression of noggin in and around newly born striatal cells promotes oligodendrogenesis rather than neurogenesis. Cells over-expressing noggin-GFP frequently had a polarized distribution of processes that projected into the striatal white matter tracts and cell bodies located around or just inside of these fibre tracts. These cells also more frequently co-express proteins indicative of oligodendroglial phenotype (PDGF-α, APC-7 and CNPase) relative to cells transduced to express GFP alone.

A previous in-vitro study has suggested the presence of a common progenitor for both GABAergic neurons and oligodendrocytes in the forebrain SVZ during development, and that the generation of these two differentiated cell populations is distinguished by spatial and temporal modulations of BMP signalling through BMP ligands such as noggin (Yung et al., 2002). It has also been shown that forebrain oligodendrocyte progenitors are born within the ventral aspect of the SVZ during embryonic development and migrate to their final destination while undergoing proliferation and differentiation to generate mature myelin producing cells (Hardy and Reynolds, 1991; Warrington et al., 1992; Woodruff et al., 2001). These reports suggest that noggin has the potential to influence the generation of both neurons and oligodendrocytes from developing forebrain progenitors. In the adult brain, while noggin continues to play a role in the generation of neurons within the SVZ (Lim et al., 2000), our present results show that antagonism of BMP signalling through noggin in cells born in the adjacent striatal parenchyma does not result in neuronal differentiation. This is consistent with the idea that noggin may create a permissive environment for neurogenesis in the adult SVZ but may not in itself be instructive for neuronal specification. Furthermore, while the SVZ is known to contain a resident population of multipotent stem cells, less is known about the plasticity of cells that divide in the striatal parenchyma. Both astrocytes and microglia are known to divide in the adult parenchyma in response to injury and, given the predominant glial phenotype of newborn cells in response to 6-OHDA injury seen here, it is quite likely that a significant fraction of these cells may well be glia to begin with. We also observed the generation of a small number of oligodendrocytes in response to injury. This is consistent with previous reports of injury induced oligodendrogliaogenesis in a neonatal model of hypoxia (Zaidi et al., 2004). Interestingly, we found that noggin over-expression in the striatum shifts the spectrum of new-born glial cell types that divide following 6-OHDA lesion in favour of a significantly larger proportion of cells with an oligodendroglial phenotype. These noggin-GFP transduced cells displayed morphological and molecular features of fully differentiated oligodendrocytes and were closely associated with myelinated fibre bundles of the internal capsule. This finding highlights that antagonism of BMP signalling may be a useful strategy for promoting oligodendrogliaogenesis in the adult brain and is consistent with a previous study reporting that there is evidence from studies that over-expression of BMP-4 under the neuron-specific enolase promoter, NSE, decreases oligodendrogial lineage commitment during forebrain development (Gomes et al., 2003). Taken together, these data suggest that forebrain parenchymal progenitors have the potential to generate new oligodendroglia in response to brain injury and that this phenomenon may be potentiated through antagonism of BMP signalling.

**Effects of Ngn2 over-expression**

We also over-expressed Ngn2 in striatal parenchymal progenitors with the aim of promoting neurogenesis in response to the 6-OHDA lesion. One week after Ngn2 gene delivery some of the transduced (GFP positive) cells had the morphological profile of immature neurons, however, many of them had unhealthy profiles with punctate or disrupted processes and expressed caspase-3, suggesting that they were undergoing apoptotic cell death. By the end of the second week there were significantly fewer GFP-positive cells with neuronal characteristics, indicating that few of the immature GFP-positive cells seen at the earlier time-point had survived. Newly born cells with immature neuronal morphology did not co-express neuronal proteins such as β-III tubulin, Hu, or DCX. A rare few did express PBX, a protein that has been shown to co-localise with the DLX protein in striatal neuron progenitors of the lateral ganglionic eminence (LGE) during embryonic development (Toresson et al., 2000). Thus, it appears that while constitutive over-expression of Ngn2 in new-born cells in the adult striatum may initiate some of the genetic programming involved in initial specification of neuronal fate and acquisition of immature neuronal features, this is not sufficient for terminal neuronal differentiation and survival of the transduced cells under the conditions reported here. A critical factor may well be the persistent over-expression of Ngn2 due to the use of a constitutive promoter in our experiments. This is notably inconsistent with the expression pattern of Ngn2 in developing neurons during embryogenesis, whereby Ngn2 is transiently expressed in immature neuroblasts and subsequently down-regulated at the time of cell-cycle exist such that it is absent in post-mitotic neurons (Bertrand et al., 2002; Thompson et al., 2006). Furthermore, the idea that persistent Ngn2 expression may prohibit the survival of newly developing neurons is consistent with a previous study reporting that retroviral over-expression of Ngn1, Ngn2 and Mash1 under constitutive promoters in cortical progenitors during late embryonic development had the potential to initially promote neurogenesis during a gliogenic phase in the developing cortex,
however, the transduced cells did not survive at later time-points (Cai et al., 2000).

In summary, we report here that retroviral gene delivery is a useful technique for selectively targeting cells in the adult brain that divide in response to local cell death. In particular, we wish to highlight the utility of GFP in this context in that it provides a powerful tool for characterising the phenotypic profile of dividing cells in vivo by providing fine morphological detail. Furthermore, we report that retroviral gene delivery has the potential to alter the phenotypic fate of these newly born cells. While this approach may one day facilitate ‘self-repair’ of the brain in response to injury through the generation of cells that can contribute to functional recovery, the success of such a strategy depends on the continued development of our understanding into the complex signalling patterns that govern the phenotypic specification and differentiation of dividing cells in the adult brain.

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