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The proliferative capacity of the subventricular zone is maintained in the parkinsonian brain

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There are many indications that neurogenesis is impaired in Parkinson's disease, which might be due to a lack of dopamine in the subventricular zone. An impairment in neurogenesis may have negative consequences for the development of new therapeutic approaches in Parkinson's disease, as neural stem cells are a potential source for endogenous repair. In this study, we examined the subventricular zone of 10 patients with Parkinson's disease and 10 age- and sex-matched controls for proliferation and neural stem cell numbers. We also included five cases with incidental Lewy body disease, which showed Parkinson's disease pathology but no clinical symptoms and thus did not receive dopaminergic treatment. We quantified the neural stem cell number and proliferative capacity in the subventricular zone of these three donor groups. We found subventricular neural stem cells in each donor, with a high variation in number. We did not observe significant differences in neural stem cell number or in proliferation between the groups. Additionally, we were able to culture neural stem cells from post-mortem brain of several patients with Parkinson's disease, confirming the presence of viable neural stem cells in these brains. We have also examined the subventricular zone of a chronic, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinson's disease mouse model, and again found no effect of dopaminergic denervation on precursor proliferation. Lastly, we investigated the proliferation capacity of two different human neural stem cell lines in response to dopamine. Both cell lines did not respond with a change in proliferation to treatment with dopamine agonists and an antagonist. In summary, the adult neural stem cell pool in the subventricular zone was not clearly affected in the human parkinsonian brain or a Parkinson's disease mouse model. Furthermore, we did not find evidence that dopamine has a direct effect on human neural stem cell proliferation *in vitro*. Thus, we conclude that the number of adult neural stem cells is probably not diminished in the parkinsonian brain and that dopamine depletion most likely has no effect on human neural stem cells.

Keywords: neural stem cells; Parkinson's disease; adult neurogenesis

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Abbreviations: GFAP = glial fibrillary acidic protein; L-DOPA = L-3,4-dihydroxyphenylalanine; MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PCNA = proliferating cell nuclear antigen; pHH3 = phosphohistone H3

Introduction

Parkinson's disease is a progressive neurological disorder, which is classically defined by motor abnormalities such as tremor and postural instability. The primary cause of these motor symptoms is the loss of dopaminergic neurons in the substantia nigra. A further neuropathological hallmark of the disease is the presence of α-synuclein-positive Lewy bodies and dystrophic Lewy neurites throughout the brain, which initially occur in the vagal nerve and olfactory bulb, and thereafter spread to other nuclei and cortical areas (Braak *et al.*, 2003). Lewy body pathology can be observed in the preclinical phase of Parkinson's disease (reviewed in Gaig and Tolosa, 2009) in the brain stem and olfactory bulb (Braak *et al.*, 2003); these preclinical cases are classified as having incidental Lewy body disease (Dickson *et al*., 2008). It has been suggested that the olfactory bulb pathology is related to olfactory dysfunction, which is a common finding in patients with Parkinson's disease (Hawkes *et al*., 1997) and their relatives (Berendse *et al*., 2001). There are studies that hypothesize that hyposmia is due to a doubling of the number of dopaminergic neurons in the periglomerular layer of the olfactory bulb (Huisman *et al*., 2004) or a reduction in olfactory bulb volume and neuronal numbers in the anterior olfactory nucleus (Pearce *et al*., 1995). These results, however, have to be taken with caution, because contradicting results have been found for both these studies (Mueller *et al*., 2005; Huisman *et al*., 2008).

Currently, treatment strategies are based on increasing dopamine levels using L-DOPA (L-3,4-dihydroxyphenylalanine; reviewed in Korecka *et al.*, 2007), which will alleviate the motor problems, but unfortunately will not cure the disease or stop the degenerative process. The caudate nucleus and putamen are close to the main neurogenic area in the subventricular zone of the adult human brain (Sanai *et al.*, 2004), which contains neural stem cells up to an advanced age (van den Berge *et al.*, 2010). Thus, the subventricular zone contains a source of endogenous stem cells, which potentially can be differentiated into dopaminergic neurons. These new dopaminergic cells could hopefully be engaged as a novel therapy to treat patients with Parkinson's disease.

The subventricular zone in the adult human brain generates new neurons throughout life and is located along the lateral ventricle wall (Quinones-Hinojosa *et al.*, 2006). It contains astrocytic neural stem cells (Sanai *et al.*, 2004), also called B cells, which specifically express glial fibrillary acidic protein (GFAP)-δ (Roelofs *et al*., 2005; van den Berge *et al.*, 2010). These neural stem cells form fast-amplifying C cells (Doetsch *et al.*, 1997), which give rise to the A cells. The A cells migrate through the rostral migratory stream and differentiate into new interneurons in the olfactory bulb (Lois *et al.*, 1996; Curtis *et al.*, 2007). The human subventricular zone can react to brain injury, such as stroke, by upregulating proliferation and inducing migration of neuroblasts (Marti-Fabregas *et al.*, 2010), which implies that the neural stem cells can indeed be targeted for future brain repair strategies. As the subventricular zone stem cells might be able to produce new dopaminergic neurons for either the substantia nigra or the striatum, they might be recruited to compensate for the dopamine loss (reviewed in Arias-Carrion *et al.*, 2007; Geraerts *et al.*, 2007).

The proliferation of subventricular zone precursors, i.e. neural stem cells and progenitor cells, is regulated by many transcription factors (e.g. Pax6 and Bmi-1) and growth factors (e.g. epidermal growth factor and fibroblast growth factor 2), and also by dopamine (for review, see Zhao *et al.*, 2008). In Parkinson's disease, the dopaminergic innervation of the striatum is lost, which might affect the neighbouring subventricular zone, as neural precursors are found close to dopaminergic fibres originating from the substantia nigra (Höglinger *et al.*, 2004). The loss of dopamine has been shown to influence neurogenesis in several animal models of Parkinson's disease, such as the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model in rodents (Höglinger *et al.*, 2004) and macaques (Freundlieb *et al.*, 2006), the 6-hydroxydopamine model in rodents (Baker *et al.*, 2004; Höglinger *et al.*, 2004), and transgenic Parkinson's disease mouse models expressing human α-synuclein (Winner *et al*., 2004, 2008*b*). In general, the data suggest that the lack of dopamine leads to a decrease in precursor proliferation in the subventricular zone. It has been shown that dopamine can stimulate C cells to generate A cells (Höglinger *et al.*, 2004), via epidermal growth factor receptor activation (O'Keeffe *et al.*, 2009). Studies on this effect in the human brain are rare. Höglinger *et al*. (2004) provided limited data in a small group of patients with Parkinson's disease on a potential decrease in proliferation in the subventricular zone. In another study, it was demonstrated that, in the human subventricular zone, the degeneration process in Parkinson's disease leads to a decrease in the number of C cells (O'Keeffe *et al.*, 2009). The decrease in the proliferative capacity of the subventricular zone and the subsequent decrease in olfactory bulb neurogenesis, therefore, might potentially account for the loss in olfactory function that has been described in patients with Parkinson's disease.

In this study, we investigated proliferation in the subventricular zone of the human Parkinson's disease brain, in a mouse Parkinson's disease model and in human neural stem cell cultures. Our study is the first to compare extensively the proliferation capacity in the human subventricular zone of a large cohort of non-demented control subjects, patients with Parkinson's disease and cases with incidental Lewy body disease. We observed that the proliferative capacity of the subventricular zone was not clearly affected in the parkinsonian brain, which is in contrast with earlier data in animal studies and the single limited study in the human brain. In addition, dopamine did not markedly influence proliferation of human neural stem cells in culture. Our finding opens up new possibilities for developing strategies to stimulate these cells to migrate into the striatal area and to differentiate into dopaminergic neurons.

Materials and methods

Post-mortem human brain material

Formalin fixed, paraffin-embedded tissue from the striatum, containing the neighbouring subventricular zone, of 10 non-demented control, 10 clinically and pathologically confirmed Parkinson's disease and five pathologically confirmed cases with incidental Lewy body disease was obtained from the Netherlands Brain Bank. We also obtained the substantia nigra from five of these controls, five cases with incidental Lewy body disease and four cases with Parkinson's disease. Additionally, we obtained formalin fixed, paraffin-embedded tissue of the olfactory bulb from six non-demented controls and seven cases with Parkinson's disease. The Netherlands Brain Bank performs rapid brain autopsies, and the brain donors have given informed consent for the use of the tissue and for accessing the extensive neuropathological and clinical information for scientific research (Huitinga *et al.*, 2008). Clinico-pathological information of all donors can be found in Table 1.

Human post-mortem neurosphere cultures

Neurosphere cultures from the subventricular zone of patients with Parkinson's disease (for donor information, see Table 1) were established, differentiated and stained as described previously (Leonard *et al.*, 2009; van den Berge *et al.*, 2010). In brief, the cell pellet obtained after dissociation and purification was taken up in serum-free medium containing epidermal growth factor and fibroblast growth factor 2 (both from Tebu-Bio). For differentiation, neurospheres were replated in complete DMEM (Dulbecco's modified Eagle's medium) on coated coverslips. For immunostaining, the coverslips were incubated with primary antibodies (see Supplementary Table 1) and species-appropriate secondary antibodies.

MPTP administration in mice

We used chronic MPTP administration to model Parkinson's disease in 12-week-old C57/BL6 male mice, as described previously (Petroske *et al.*, 2001). Animals were housed in an enriched cage environment, with *ad libitum* food and water. The experiment was approved by the Institutional Animal Care and Use Committee of the Royal Netherlands Academy of Arts and Sciences. Two groups, of six and eight animals, received subcutaneous injections of either 25 mg/kg MPTP (Sigma-Aldrich) or saline bi-weekly for 5 weeks. Probenecid (250 mg/kg; Sigma) intraperitoneally was used as an adjuvant to decrease MPTP clearance. Animals were given a lethal dose of Nembutal (A.U.V.) 3.5 days after the final injection, and were then transcardially perfused with saline, followed by 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4). The brain was dissected and post-fixed overnight in 4% paraformaldehyde.

Human neural stem cell cultures

We cultured an immortalized human neural stem cell line (De Filippis *et al.*, 2007) in Euromed-N medium (Euroclone, S.p.A.) containing 25 μg/ml insulin, 100 μg/ml transferrin, 6.3 ng/ml progesterone, 9.6 μg/ml putrescine, 520 ng/ml selenite (N2 supplement, all from Sigma), 20 ng/ml epidermal growth factor and 10 ng/ml fibroblast growth factor 2 (both from Tebu-Bio). After dissociation, cells were plated in a 24-well plate with or without growth factors in combination with dopamine agonists or antagonists. The final concentrations were: 5 and 10 μM of dopamine, and 1 μM of the D1 receptor agonist (±)-SKF-38393 hydrochloride, the D2-like agonist 2-bromo-α-ergocryptine methanesulphonate and the D2-like antagonist (±)-Sulpiride (all from Sigma). After 5 days, neurospheres were photographed and the number of spheres in a well and the mean diameter of the spheres were determined in each condition. This experiment was repeated in three independent experiments with $n = 2$ for each treatment condition.

Additionally, we cultured the foetal human neural precursor cell line CB660 (Sun *et al.*, 2008) in Euromed-N medium containing N2 supplement, 20 ng/ml epidermal growth factor and 20 ng/ml fibroblast growth factor 2 on laminin-coated flasks. For the experiment, cells were plated in a 24-well plate with or without growth factors in combination with 5 and 10 μM of dopamine. After 16 h, 5 μM bromodeoxyuridine (Sigma) was added for 2 h, after which cells were fixed for 20 min in 3.7% formaldehyde solution (Sigma) in phosphate-buffered saline (50 mM potassium phosphate, 150 mM NaCl; pH 7.2) and stained for bromodeoxyuridine (Supplementary material).

Quantitative polymerase chain reaction

Cell pellets of the immortalized human neural stem cell line were collected, and we extracted RNA and synthesized complementary DNA as described previously (Middeldorp *et al.*, 2009). Additionally, we collected fresh human subventricular zone material from a control donor at autopsy. Of both the immortalized human neural stem cells and the subventricular zone, we analysed the expression level of the dopamine receptors with quantitative reverse transcription polymerase chain reaction with the SYBR green method (Middeldorp *et al.*, 2009) (D1R forward primer: AACCACATTTCTGGCCATTT, reverse primer: TCCC TTATCTATCAGTTTCTGCTGT; D2R forward primer: GGAAATTCAGC AGGATTCACTG, reverse primer: ATGCTGATGGCACACAAGTTC; D3R forward primer: TTTGTCACCCTGGATGTCATG, reverse primer: GGCA CAGAGATTAAGGATGCTG; D4R forward primer: CCTTCTTCGTGG TGCACAT, reverse primer: AACTCGGCGTTGAAGACAGT; and D5R forward primer: TCCACAAGGAAATCGCAGCT, reverse primer: AAC ATGCGATCGAAAGGACC).

High-performance liquid chromatography

We collected immortalized human neural stem cell medium at different time points after adding dopamine to the culture medium. The dopamine concentration of these samples was analysed using a HPLC ALEXYS 100 2D system equipped with electrochemical detection (DECADE II) from ANTEC Leyden. Samples of 5 μl were injected to analyse dopamine on an ALF-105 (50 × 1 mm, 3 μm C18) column (ANTEC Leyden). The mobile phase composition was: 50 mM phosphoric acid 85%, 8 mM KCl, 12.5% methanol and 500 mg/l octanesulphonic acid, pH 6.0. Separation was performed at 35°C, the electrochemical potentials were set at 350 mV against an Ag/AgCl reference in the ISAAC electrochemical cell. The signals were analysed using Clarity (2.6.4.402) software. The detection limit in a 5 μl sample at a signal to noise ratio 3 was 0.02 nM.

Immunohistochemistry

Immunostaining was performed according to a fairly standard protocol, described in full in the Supplementary material. Briefly, epitope

Table 1 Clinicopathological data of donors

NBB number	Material	Diagnosis	Sex	Age (years)	Braak AD[a]		Braak PD[b]	PMD (h:min)	pH CSF	Brain weight (g)	PD duration (years)	ApoE	Intoxication/ medication	Cause of death
1993-015	Subventricular zone and substantia nigra	C	M	75	1			04:15	7.24	1252	–	33	?	Respiratory insufficiency
1997-143	Subventricular zone and substantia nigra	C	M	79	1	B	PD-0	06:00	6.51	1392	–	33	Cort, Benzo	Extensive metastases
1998-126	Subventricular zone and substantia nigra	C	M	71	2		PD-0	06:00	6.54	1385	–	43	Cort, SSRI, Benzo, α-psych	Respiratory insufficiency?
1999-052	Subventricular zone and substantia nigra	C	F	79	2	B		05:30	6.21	1325	–	43	Cort, Benzo	Respiratory insufficiency
2001-021	Subventricular zone	C	M	82	1	O	PD-0	07:40	6.07	1373	–	33	Nico, Cort	Heart attack
2001-028	Subventricular zone	C	F	78	1	A	PD-0	04:50	6.4	1250	–	33	Nico, Cort, Benzo	Myocardial infarction
2001-046	Subventricular zone	C	M	88	1	C	PD-0	07:25	6.5	1228	–	33	Nico, Cort, Benzo	Legal euthanasia
2003-040	Subventricular zone	C	F	73	0	B	PD-0	04:00	6.47	1360	–	32	Cort	Lung fibrosis
2003-094	Subventricular zone	C	M	85	2	C		04:35	6.62	1374	–		Nico, Benzo	Cardiac arrest/ dehydration/ cachexia
2005-019	Olfactory bulb	C	M	74	3	C	PD-0	05:00	6.7	1125	–	43	Nico, Cort, Benzo, α-psych	Bronchus carcinoma
2005-060	Olfactory bulb	C	M	91	1	B	PD-1	08:00	6.26	1243	–	33	-	Cardiac decompensation
2005-061	Olfactory bulb	C	F	93	2	O	PD-0	05:50		1145	–	33	Cort, Benzo	Cachexia
2005-083	Subventricular zone	C	F	85	1	B	PD-0	05:00	6.72	1257	–	33	Cort, Benzo	Multi organ failure after a ruptured abdominal aneurysm
2006-080	Olfactory bulb and substantia nigra	C	F	89	2	B	PD-0	06:25	6.46	1210	–	32	Cort, Benzo	Old age, possible ruptured abdominal aneurysm
2007-007	Olfactory bulb	C	M	84	1	A	PD-0	05:35	6.98	1457	–	33	Cort, Benzo	Heart failure
2007-014	Olfactory bulb	C	M	86	2	B		04:00		1250	–		Nico, Cort, Benzo	Respiratory insufficiency
2002-037	Subventricular zone and substantia nigra	iLBD	F	91	3	B	PD-5[c]	07:05	6.1	1065	–	33	Benzo, α-psych	Heart failure
2005-014	Subventricular zone and substantia nigra	iLBD	F	86	3	B	PD-3	06:25	7.07	1127	–	33	Cort, Benzo	Heart attack?
2005-063	Subventricular zone and substantia nigra	iLBD	F	93	2	O	PD-3	04:00	6.64	1316	–	33	Benzo	Heart failure
2006-003	Subventricular zone and substantia nigra	iLBD	M	98	2	O	PD-3	10:46	6.35	1200	–	32	-	Unknown
2007-056	Subventricular zone and substantia nigra	iLBD	M	79	2	B	PD-6[c]	09:00	6.15	1295	–	33	Nico, Benzo, α-psych	Pneumonia and metastasised kidney carcinoma
2000-034	Subventricular zone	PD	M	86	2	A	PD-5	08:30	6.52	1178	17	32	L-DOPA, Cort	Unknown
2002-003	Subventricular zone	PD	F	75	1	B	PD-5	05:00	6.52	1218	21	43	L-DOPA, SSRI, Benzo, α-psych	Legal euthanasia
2002-011	Subventricular zone	PD	F	79	1	O	PD-4	05:45	6.37	1203	9	43	L-DOPA, Benzo	Myocardial infarction
2002-013	Subventricular zone	PD	F	80	1		PD-3	05:30	6.09	1254	32	43	L-DOPA, Cort, Benzo	Cachexia

(continued)

Table 1. Continued

NBB number	Material	Diagnosis	Sex	Age (years)	Braak AD[a]		Braak PD[b]	PMD (h:min)	pH CSF	Brain weight (g)	PD duration (years)	ApoE	Intoxication/ medication	Cause of death
2002-022	Subventricular zone and substantia nigra	PD	M	84	2	B	PD-5	05:10	6.8	1198	10	33	L-DOPA, Benzo	Endocarditis/heart failure
2003-064	Subventricular zone	PD	M	83	1	B	PD-4[d]	05:45	6.14	1217	4	43	L-DOPA, SSRI	Respiratory insufficiency
2004-059	Subventricular zone	PD	M	74	1	O	PD-5	02:50	6.55	1259	21	33	Benzo	Dehydration, uremic coma, possible CVA
2004-080	Subventricular zone and olfactory bulb	PD	M	71	1	A	PD-6	05:50	6.27	1552	39	43	Nico, L-DOPA, Benzo, α-psych	Respiratory failure
2005-069	Subventricular zone, olfactory bulb and substantia nigra	PD	M	77	1	O	PD-5	08:05	6.45	1380	13	32	L-DOPA, Benzo	Pneumonia leading to septic shock
2005-080	Olfactory bulb	PD	M	73	1	A	PD-3	06:35	6.28	1572	3	33	L-DOPA, SSRI, Benzo, α-psych	Aspiration pneumonia
2006-002	Subventricular zone, olfactory bulb and substantia nigra	PD	F	84	2	B	PD-5	07:25	6.85	1244	12	33	L-DOPA, Cort, SSRI, Benzo, α-psych	Unknown
2006-062	Olfactory bulb	PD	M	87	1	B	PD-5	03:40	6.33	1205	21		L-DOPA, Benzo	Cachexia and dehydration
2007-008	Olfactory bulb and substantia nigra	PD	M	80	1	B	PD-6	07:05	6.34	1612	6	43	L-DOPA, Benzo, α-psych	Unknown
2007-029	Olfactory bulb	PD	M	67	1	O	PD-6	04:55	6.4	1257	18	32	Nico, L-DOPA, Cort, Benzo	Sepsis by pneumonia
2010-044	Neurospheres	LBV	F	71	3	C	PD-6	05:40	6.82	1413	3		Nico, L-DOPA, Benzo	Dehydration and pneumonia
2010-052	Neurospheres	PDD	M	80	2	B	PD-5	05:30	6.29	1325	13		L-DOPA, Benzo, α-psych	Sudden death probably by heart failure

a Braak stage AD (Alzheimer's disease) is a score for AD pathology, referring to tau pathology (0 = no pathology up to 6 = severe pathology; Braak and Braak, 1991) and amyloid pathology (O = no amyloid deposits; A, B and C represent increasing amyloid deposits; Thal *et al.*, 2000).
b Braak stage PD (Parkinson's disease) is a scale for α-synuclein pathology (0 = no pathology up to 6 = severe pathology; Braak *et al.*, 2003).
c Atypical pattern of α-synuclein pathology in iLBD since brainstem, limbic and neocortical regions are affected.
d Amygdala predominant case (Alafuzoff *et al.*, 2009).
α-psych = atypical antipsychotics; ApoE = Apolipoprotein E genotype; Benzo = benzodiazepines; C = non-demented control; Cort = corticosteroids; CVA = cerebrovascular accident; iLBD = incidental Lewy body disease; LBV = Lewy Body variant; M/F = male/female; NBB = Netherlands Brain Bank; Nico = nicotine; PD = Parkinson's disease; PDD = PD with dementia; pH CSF indicates the agonal state of the donor (Ravid *et al.*, 1992); PMD = post-mortem delay.

retrieval was performed in a steamer and a serum-blocking step was used before incubating with the primary antibody (Supplementary Table 1) overnight. The antibodies were visualized using biotinylated secondary antibodies, Avidin Biotin Complex and diaminobenzidine, or fluorescently labelled secondary antibodies.

Image acquisition and analysis

Details about image acquisition and quantification of the substantia nigra dopaminergic cell count and different immunostainings can be found in the Supplementary material.

Results

Selection of controls and cases with Parkinson's disease and incidental Lewy body disease

The control and Parkinson's disease cases were matched for sex, age, post-mortem delay and brain weight. In addition, we selected only cases with a low Braak score for Alzheimer's disease tau pathology (Braak and Braak, 1991), to prevent the effect of a mixed pathology on our quantification. The cases with incidental Lewy body disease in our study were selected based on the neuropathological reports mentioning a Braak α-synuclein stage of three or higher, without any clinical history of Parkinson's disease. These cases had a significantly higher mean age and higher tau scores. To study the effect of striatal dopamine loss on the olfactory bulb, we also obtained the olfactory bulb from six non-demented control subjects and seven cases of Parkinson's disease, which were matched on sex and post-mortem delay. Unfortunately, we could not obtain olfactory bulbs from the same donors from whom we had subventricular zone material, because the olfactory bulb is not dissected at every Netherlands Brain Bank autopsy. We confirmed that the Parkinson's disease group had dopaminergic cell loss by quantifying the density of dopaminergic neurons in the substantia nigra of five control, five incidental Lewy body disease and four Parkinson's disease cases (Fig. 1A–D). There was a very significant decrease in neuromelanin-containing cells in the substantia nigra between the groups ($P = 0.007$; median value = 7352 cells/mm^3 for controls, 5676 cells/mm^3 for cases with incidental Lewy body

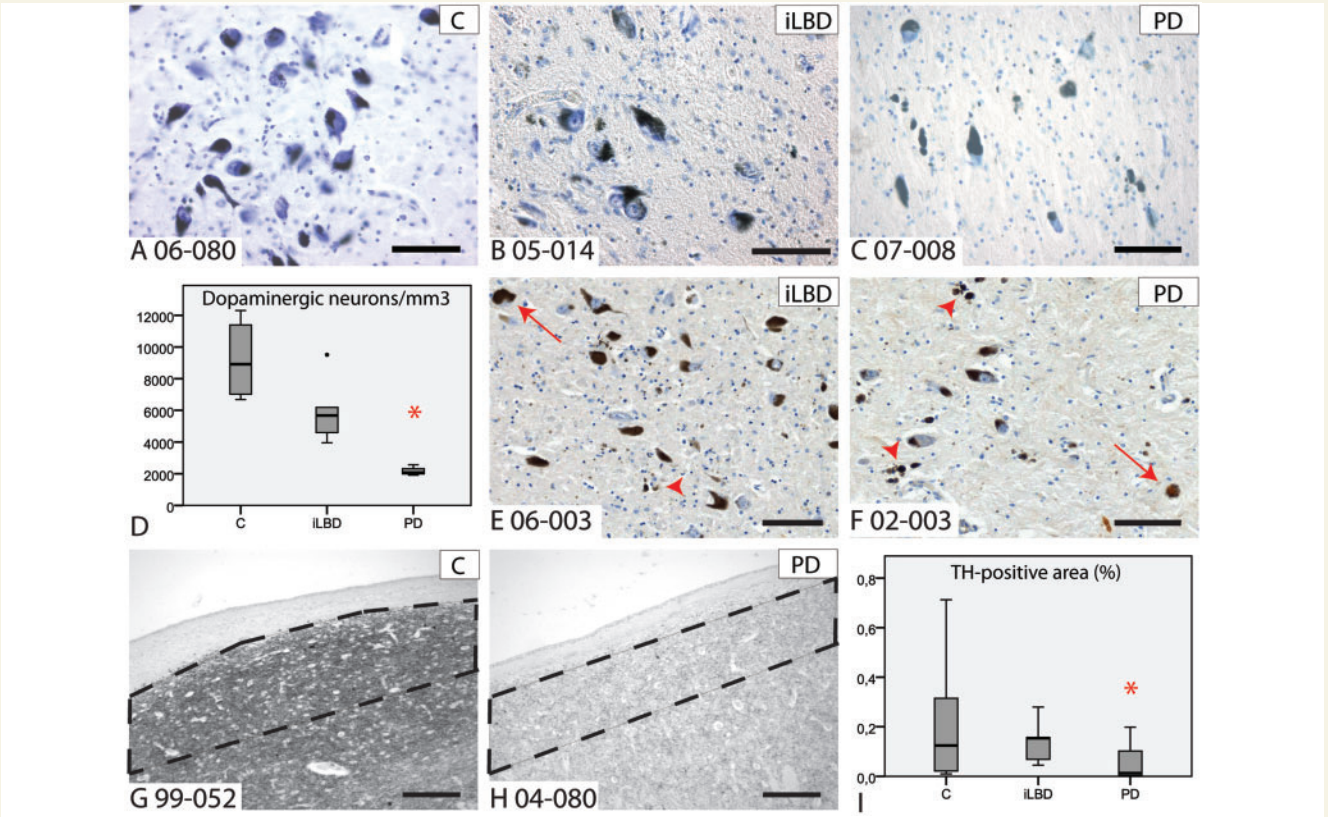

Figure 1 Substantia nigra pathology and tyrosine hydroxylase (TH) expression in the subventricular zone. (**A–C**) Nissl staining of the substantia nigra of a control (**A**), incidental Lewy body disease (iLBD) (**B**) and Parkinson's disease (PD) (**C**) donor with dopaminergic neurons in brown. (**D**) Box plot of the density of neuromelanin-containing neurons in the substantia nigra of five control subjects, five cases with incidental Lewy body disease and four cases with Parkinson's disease. (**E–F**) α-synuclein pathology in an incidental Lewy body disease (**D**) and Parkinson's disease (**E**) donor; Lewy bodies are indicated by arrows, Lewy neurites by arrowheads. (**G–H**) Examples of tyrosine hydroxylase staining in the striatum underneath the subventricular zone of a control (**G**) and a Parkinson's disease case (**H**). The dashed line indicates the area that was measured. (**I**) Box plot of the tyrosine hydroxylase expression in the area underneath the subventricular zone of 10 control, five incidental Lewy body disease and 10 Parkinson's disease cases. Scale bars indicate 100 µm.

disease and 1989 cells/mm^3 for cases with Parkinson's disease). The decrease in neuromelanin-containing cells in the cases with incidental Lewy body disease was not significant compared with the controls, but showed a strong trend ($P = 0.056$). We also visualized Lewy body pathology in the substantia nigra by α-synuclein staining; incidental Lewy body disease (Fig. 1E) and Parkinson's disease (Fig. 1F) donors showed α-synuclein-positive Lewy bodies and neurites in the substantia nigra. Additionally, we quantified tyrosine hydroxylase expression in the striatal area immediately underneath the subventricular zone (Fig. 1G–I), to confirm that the dopaminergic input from the substantia nigra was decreased in this area. We showed a significant difference in tyrosine hydroxylase expression between the groups ($P = 0.044$; median value = 12.41% for controls, 15.39% for cases with incidental Lewy body disease and 1.27% for cases with Parkinson's disease). The decrease in the tyrosine hydroxylase-positive area in cases with Parkinson's disease as compared with controls was highly significant ($P = 0.023$). The cases with incidental Lewy body disease did not show a significant decrease in tyrosine hydroxylase expression in the area underneath the subventricular zone, but there was wide variation in this group. Tyrosine hydroxylase staining under the subventricular zone did correlate very strongly with the presence of dopaminergic cells in the substantia nigra measured in the same cases (correlation coefficient = 0.839, $P = 0.001$). The subventricular zone was also checked for expression of dopamine receptors, to confirm that dopamine depletion could have an effect on the cells in this area. We could detect messenger RNA of the D1, D2, D3, D4 and D5 receptors in post-mortem freshly isolated subventricular zone tissue from adult human brain (data not shown).

Proliferation capacity of subventricular zone precursor cells is unaffected in the brains of patients with Parkinson's disease

We analysed the proliferation capacity in the human subventricular zone near the striatum in controls and patients with incidental Lewy body disease and patients with Parkinson's disease, using two independent and established proliferation markers, proliferating cell nuclear antigen (PCNA) and phosphohistone H3 (pHH3). PCNA is produced during the S-phase of the cell cycle and has a protein half-life of 20 h, which is ~10 times longer than the half-life of other cell cycle markers (Eisch and Mandyam, 2007), whereas phosphorylation of histone H3 is seen during G2 and M-phase, and is tightly regulated during the cell cycle (Juan *et al.*, 1998). We clearly observed PCNA-expressing cells, and therefore cycling cells, in patients with Parkinson's disease and in non-demented controls (Fig. 2A–F). As seen before, all ependymal cells were PCNA-positive and many PCNA-positive cells were observed in the astrocytic ribbon. We excluded the ependymal layer from our analysis. The variability between the individuals within the groups was high (Fig. 2A, C, D and F) and the PCNA-positive surface area ranged from 1.96 to 10.40% of the subventricular zone in the controls, and 0.83 to 9.24% in the cases with Parkinson's disease. A similar variability between and within the patient groups was observed in pHH3 cell numbers (Fig. 2I; Fig. 2H shows a typical example of pHH3-positive cells). The number of cells varied between 0 and 28.5/mm subventricular zone. Automated and unbiased quantification of PCNA expression (Fig. 2G) in 10 controls and 10 cases with Parkinson's disease confirmed that there was no difference in PCNA expression between controls (median PCNA-positive area = 4.48%) and cases with Parkinson's disease (median = 4.29%). The same was seen for pHH3 cell numbers per millimetre subventricular zone, which did not significantly differ between the patient groups (median = 8.45 in controls; 4.63 in cases with Parkinson's disease; see Fig. 2I). To exclude compensation of a possible effect by dopamine replacement therapy in the Parkinson's disease group, we included a group of cases with incidental Lewy body disease. Also in this group, proliferating cells in the subventricular zone were clearly present (Fig. 2B and E), with a similar variability as in the Parkinson's disease and control cases (ranging from 2.81 to 7.71%), and no apparent change in proliferation compared with these groups (Fig. 2G; median PCNA-positive area = 6.75%).

In addition to the diagnosis of Parkinson's disease, we also performed an extensive analysis of the medical and pathological information that was available of the donors to identify potential other confounding factors that could affect the proliferation capacity of neural stem cells and/or precursors in the subventricular zone proliferation (Table 1). These factors included age, sex, Braak α-synuclein score (Braak *et al.*, 2003) and smoking habits. Furthermore, the potential effect of the use of medication that might influence proliferation in the subventricular zone was studied in the last 5 years of life of the donors. We searched patient files for selective serotonin reuptake inhibitors and other antidepressant drugs, benzodiazepines and corticosteroids, as these have all been implicated to affect neurogenesis. Our analysis revealed that none of these variables could account for the variation observed in our donor cohort. We did not observe any correlation between proliferation and the available donor characteristics. We also found no correlation with pathological characteristics, such as Alzheimer's disease pathology in the form of amyloid-β and hyperphosphorylated tau staging.

No effect of Parkinson's disease pathogenesis on subventricular zone human neural stem cells

The PCNA and pHH3 analysis provides information on the proliferative capacity of all subventricular zone cells, i.e. neural stem cells (B cells) and transit-amplifying progenitors (C cells). In order to study whether the Parkinson's disease process affects the number of neural stem cells in the subventricular zone, we subsequently quantified the expression of the neural stem cell marker GFAP-δ in this region in non-demented controls, cases with incidental Lewy body disease and cases with Parkinson's disease (Fig. 3A–C). In comparison with our earlier studies on the expression of GFAP-δ in other subventricular zone areas in the ageing brain (van den Berge *et al.*, 2010), we

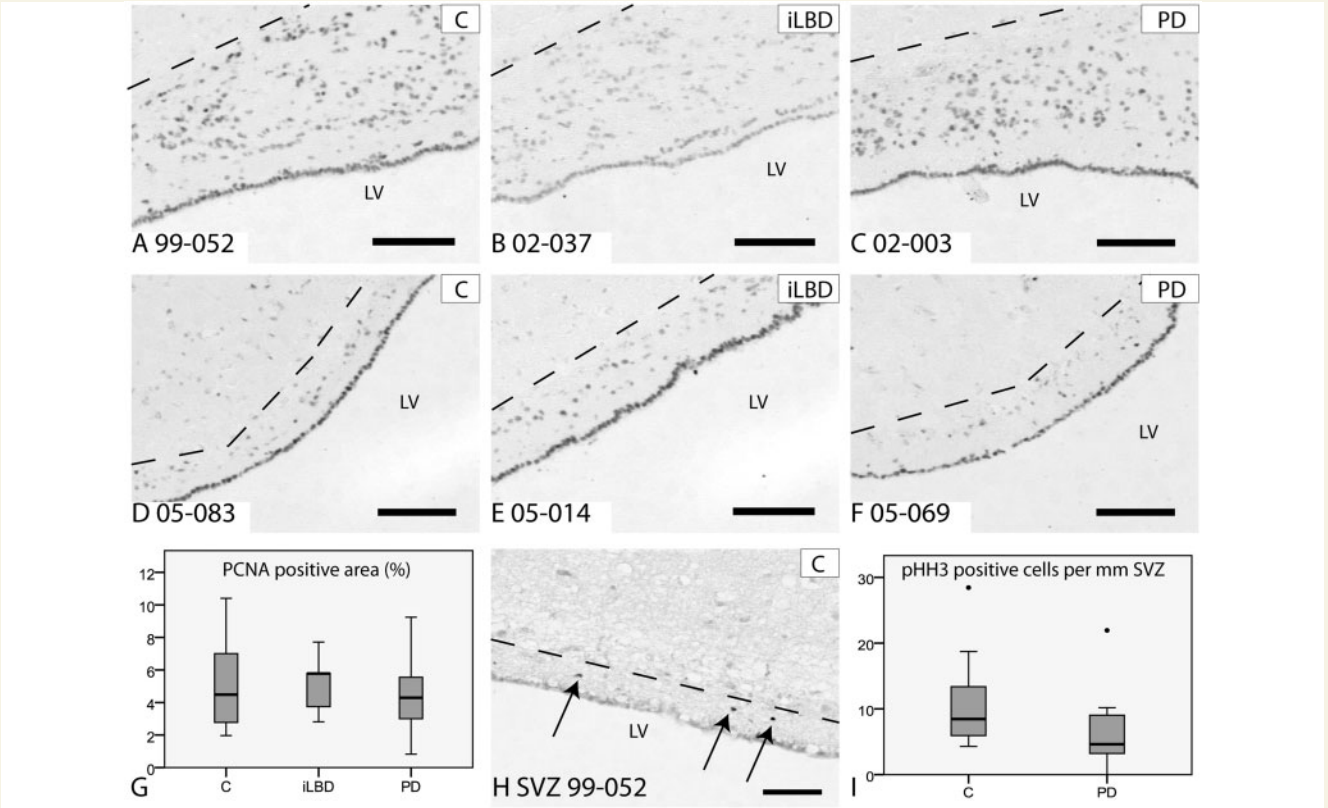

Figure 2 Proliferation in the human parkinsonian subventricular zone (SVZ). (**A–F**) Examples of high (**A–C**) and low (**D–F**) PCNA expression in the subventricular zone of control donors (**A**, **D**), incidental Lewy body disease (iLBD) donors (**B**, **E**), and patients with Parkinson's disease (PD) (**C**, **F**). (**G**) Box plot of the quantification of PCNA expression in 10 control subjects, five cases with incidental Lewy body disease and 10 cases with Parkinson's disease. (**H**) Example of pHH3 staining (*arrows*) in the subventricular zone of a control donor. (**I**) Box plot of the quantification of pHH3 cell numbers in the subventricular zone of 10 control and 10 incidental Lewy body disease donors. The subventricular zone is indicated by a dashed line; LV = lateral ventricle; scale bars indicate 100 μm in (**A–F**), 50 μm in (**H**).

observed that the width of the ribbon alongside the striatum was generally about a factor of five times thinner than in our previous study; most donors showed an astrocytic ribbon that was only one cell wide. In analogy with the PCNA and pHH3 expression, there was again a considerable variation in GFAP-δ-positive subventricular zone area between donors (Fig. 3G, ranges were 0.05–3.13% in controls, 0.77–4.41% in cases with incidental Lewy body disease and 0.13–3.77% in cases with Parkinson's disease). The expression of GFAP-δ was not significantly different between 10 control (median = 0.64%), five incidental Lewy body disease (2.76%) and 10 Parkinson's disease cases (1.55%). However, there was a trend ($P = 0.058$) towards an increase in Parkinson's disease versus control cases. In addition to the GFAP-δ expressing cells in the subventricular zone, we studied these cells in the olfactory bulb of seven patients with Parkinson's disease and six control donors. GFAP-δ expression was found in the same pattern as described before (van den Berge *et al.*, 2010). Its expression was highly variable and not significantly different between cases with Parkinson's disease (Fig. 3D–F; median = 0.41% of the olfactory bulb surface area) and control subjects (0.49%; Fig. 3H). An analysis for confounding factors affecting GFAP-δ expression in the olfactory bulb did not reveal significant correlations, excluding the fact that one single donor trait influenced our results.

Parkinson's disease brains contain viable neural precursor cells

To prove that Parkinson's disease brains indeed contain viable neural precursors, we decided to isolate these cells from the subventricular zone of post-mortem Parkinson's disease brains and culture them as neurospheres as we have described before (Leonard *et al.*, 2009). We were able to culture neurospheres from patients with Parkinson's disease (Fig. 4A) with a similar success rate as from control donors (van den Berge *et al.*, 2010). We obtained neurospheres in 40% (4/10) of the Parkinson's disease and 53% (7/13) of the control cases. More detailed information about the post-mortem delay, age, sex and medication of the patients can be found in Table 1. We were also able to differentiate the neurospheres from patients with Parkinson's disease into astrocytes, neurons and oligodendrocytes (Fig. 4B and C). These data strongly indicate that the Parkinson's disease subventricular zone still contains proliferative, multi-potent neural stem cells.

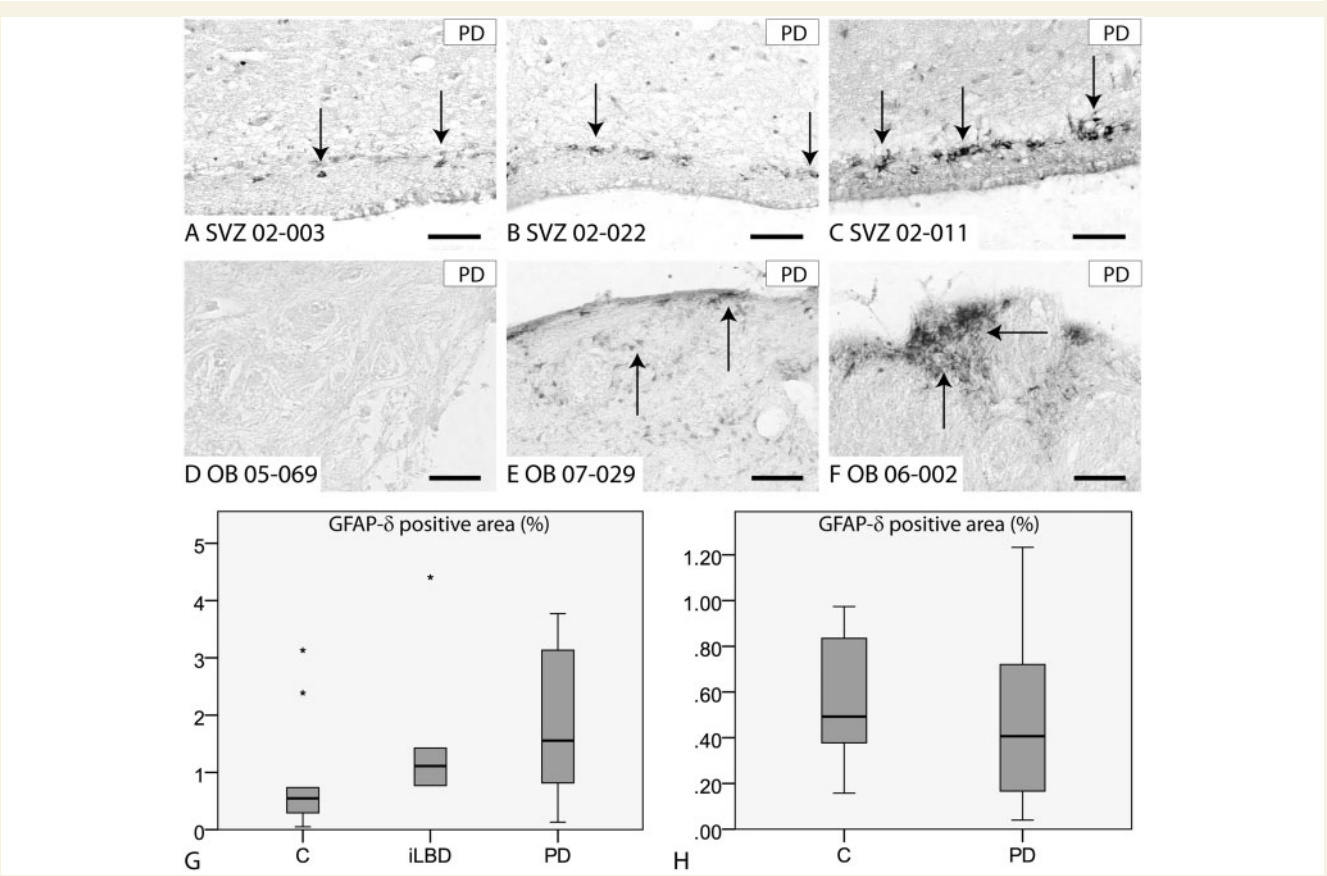

Figure 3 GFAP-δ-positive cells in the human parkinsonian subventricular zone (SVZ) and olfactory bulb (OB). (**A–C**) GFAP-δ expression (*arrows*) in the subventricular zone (SVZ) of three patients with Parkinson's disease (PD). (**D–F**) GFAP-δ expression (*arrows*) in the olfactory bulb of three patients with Parkinson's disease. (**G**) Box plot of the quantification of GFAP-δ expression in the subventricular zone of 10 control subjects, five cases with incidental Lewy body disease and 10 cases with Parkinson's disease. (**H**) Box plot of the quantification of GFAP-δ expression in the olfactory bulb of six control and seven Parkinson's disease cases. Scale bars indicate 50 μm in (**A–C**), 100 μm (**D–F**).

Chronic MPTP administration does not decrease subventricular zone proliferation in mice

To substantiate our results observed in the human parkinsonian brain, we decided to study subventricular zone proliferation in a mouse model for Parkinson's disease. Dopamine depletion was induced in mice using a previously established chronic MPTP/probenecid model, which has been described to show sustained nigrostriatal degeneration and motor problems resembling the progress of human Parkinson's disease (Petroske *et al.*, 2001). We first confirmed that we could induce dopamine depletion of the subventricular zone in these mice by quantifying striatal tyrosine hydroxylase expression, which was indeed significantly (Fig. 5D, $P < 0.000$) decreased in the MPTP-treated animals (mean intensity = 105.8 ± 2.8; Fig. 5B, *green*) compared with controls (mean intensity = 125.2 ± 2.3; Fig. 5A, *green*). We also confirmed that the substantia nigra was affected by examining tyrosine hydroxylase staining in this area (Fig. 5G and H), which had almost completely vanished in the MPTP-treated animals versus control animals. Subsequently and in analogy with our experiments in the human subventricular zone, we analysed the proliferation capacity of the subventricular zone in these mice using PCNA and pHH3 as markers. PCNA expression was found in cell nuclei in the subventricular zone, and the levels did not differ significantly (Fig. 5E; $P = 0.782$) between control (mean area percentage = 12.98 ± 0.93%; Fig. 5A, *purple*) and MPTP-treated mice (12.48 ± 1.57%; Fig. 5B, *purple*). Phosphohistone H3 cell numbers were only few in controls (1.58 ± 0.04 cells/mm subventricular zone), and these numbers were significantly increased after MPTP treatment (1.82 ± 0.09 cells/mm subventricular zone; $P = 0.021$; Fig. 5C and F, *purple*).

Proliferation of human neural stem cells is not affected by dopaminergic stimulation

To investigate whether dopamine can have an effect on proliferation of human neural stem cells, we cultured two different human neural stem cell lines and investigated proliferation of

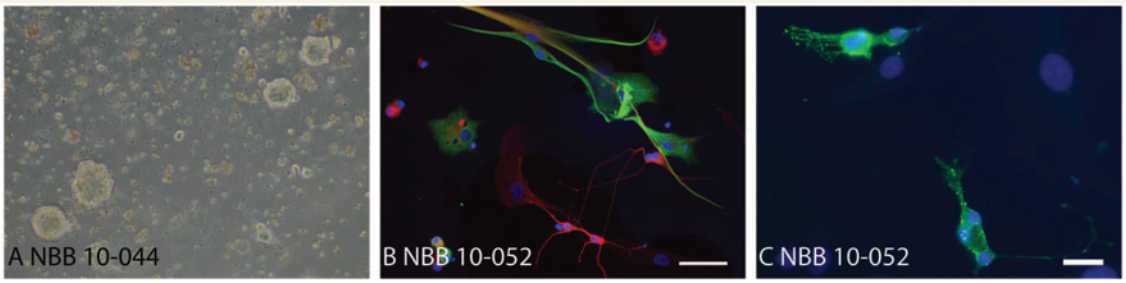

Figure 4 Neurosphere cultures from the human parkinsonian brain. (**A**) Neurospheres from a 71-year-old female patient with Parkinson's disease in culture. (**B–C**) Differentiation of neurospheres from an 80-year-old Parkinson's disease donor resulted in GFAP-positive astrocytes (**B**, *green*), β-III-tubulin-positive neurons (**B**, *red*) and galactocerebroside-positive oligodendrocytes (**C**, *green*). Blue staining is Hoechst staining for cell nuclei; scale bars indicate 50 μM in (**A**) and 25 μM in (**B**).

these cells after dopaminergic stimulation. The proliferation of immortalized human neural stem cells (Fig. 6A) was determined by measuring neurosphere diameter and number, because quantification of bromodeoxyuridine labelling was troublesome due to the 3D structure of the neurospheres. In CB660 cells, we determined the total number of cells and the bromodeoxyuridine labelling percentage (Fig. 6B). We verified that dopamine could exert effects on the immortalized human neural stem cell line by determining dopamine receptor messenger RNA levels. All dopamine receptors were detectable in the immortalized human neural stem cells (data not shown). In addition, we checked the stability of dopamine in cell culture medium by high-performance liquid chromatography, and we found a steady decline in dopamine concentration over time, with dopamine being broken down within ~18 h (extrapolated from 8 h of measuring; Fig. 6C). This breakdown was independent of the presence of cells in the medium. The immortalized human neural stem cell line did not respond to stimulation with either dopamine itself, the dopamine D1 receptor agonist SKF-38393, the D2 receptor agonist bromocryptine or the D2 receptor antagonist sulpiride (Fig. 6D and E). This lack of effect was independent of the presence or absence of growth factors in the medium. We could clearly measure the difference in growth rate between the condition with and without growth factors, showing that our assay can pick up differences in proliferation. CB660 cells also did not increase their proliferation rate after addition of dopamine (Fig. 6F and G).

Discussion

In this study, we provide evidence that the proliferative capacity in the human and mouse subventricular zone is not changed or slightly increased (pHH3 expression in the mouse) after dopaminergic denervation of the striatal subventricular zone. In addition, we show that the proliferative capacity of human neural stem cells is probably not influenced by dopamine. These data are in sharp contrast with previously published data on both the human subventricular zone and the mouse subventricular zone after MPTP treatment (Höglinger *et al.*, 2004), or 6-hydroxydopamine-induced dopamergic denervation (Baker *et al.*, 2004; Höglinger *et al.*, 2004; Winner *et al.*, 2006) and suggest that the hypothesis that dopamine stimulates proliferation of human neural precursor cells has to be reconsidered.

Our data show that the neural stem cell pool in the subventricular zone is not affected by the disease progression in our set of patients with Parkinson's disease. We could clearly show, using two established cell division markers, that the subventricular zone precursor cells are able to proliferate in elderly brains and in brains of incidental and clinical patients with Parkinson's disease. We also confirmed our earlier studies (van den Berge *et al.*, 2010), in which we found a highly variable width of the ribbon of neural stem cells in the subventricular zone. The presence of neural stem cells in Parkinson's disease brains was confirmed by their ability to form neurospheres, which could be differentiated into neurons, astrocytes and oligodendrocytes. To find out what could underlie the high variability of the thickness of the astrocytic ribbon and the number of neural stem cells we checked for confounding candidates in our patient population. We were not able to find a single factor that could account for the observed variability in precursor cell proliferation or neural stem cell numbers in the human brain. There are many factors that are known to influence proliferation and neural stem cells in the subventricular zone of rodents, and probably also play a role in the human brain. These factors include age (Ahlenius *et al.*, 2009), sex (Diaz *et al.*, 2009), stress (Hitoshi *et al.*, 2007) and nicotine administration (Belluardo *et al.*, 2008), i.e. smoking. Medication that might influence the subventricular zone cell population includes selective serotonin reuptake inhibitors and other antidepressants (Lau *et al.*, 2007; Seiji *et al.*, 2007), atypical antipsychotics (Green *et al.*, 2006), benzodiazepines (Wang *et al.*, 2003) and corticosteroids (Lau *et al.*, 2007). The factors that might influence the subventricular zone were found in multiple patients but in different combinations, creating a high variability between different donors. Corticosteroid use was more prevalent in the control cases, but it is unlikely that this affects the subventricular zone, as the effects of corticosteroids are limited to the hippocampus (Lucassen *et al.*, 2010). Furthermore, in rodents, the plasma corticosterone levels do not correlate with the number of bromodeoxyuridine-positive cells in the hippocampus, implying that glucocorticoids do not have a direct effect on cell proliferation (Mirescu *et al.*, 2004). Also, the cases were treated with synthetic glucocorticoids,

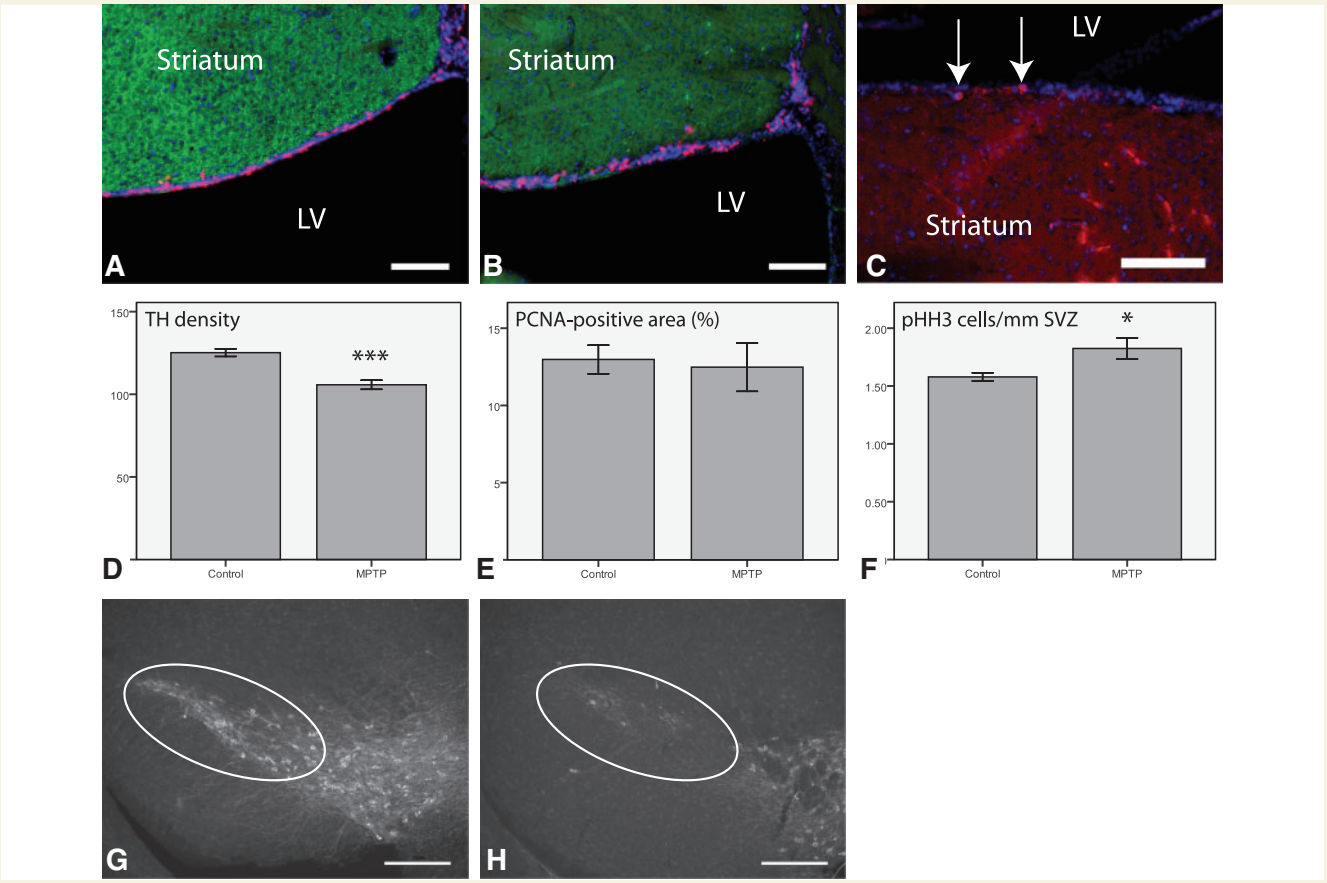

Figure 5 Proliferation after dopaminergic denervation in the mouse subventricular zone (SVZ). (**A**, **B**) PCNA expression (*red*) in the subventricular zone overlying the striatum, identified by tyrosine hydroxylase (TH) expression (*green*) in a control (**A**) and MPTP-treated mouse (**B**). (**C**) pHH3 expression (*red*, *arrows*) in the subventricular zone of an MPTP-treated mouse. (**D–F**) Quantification of tyrosine hydroxylase (**D**), PCNA (**E**) and pHH3 (**F**) expression in the subventricular zone of eight control and eight MPTP-treated mice. (**G**, **H**) Tyrosine hydroxylase staining in the substantia nigra of a control (**G**) and an MPTP-treated mouse (**H**). Blue staining is Hoechst staining for cell nuclei; LV = lateral ventricle; scale bars indicate 100 μm in (**A**, **B**), 50 μm in (**C**), 200 μm in (**G**, **H**); graphs represent mean ± SEM; $*P \leqslant 0.05$, $**P \leqslant 0.01$.

which are removed from the brain through multidrug resistance *P*-glycoprotein (Karssen *et al*., 2001), which prevents direct effects of synthetic glucocorticoids on brain cells. Besides the exposure of the donors to drugs that can modulate neurogenesis, it is highly likely that the genetic background of the individuals contributes to the variation in the number of neural stem cells. It has been shown that the genetic background in mice has an enormous influence on the level of neurogenesis in the hippocampus (Kempermann *et al.*, 2006). Furthermore, a recent publication by Poon *et al*. (2010) provides evidence that the rostral migratory system might be similarly affected by the genetic background, which makes it likely that the subventricular zone is also influenced by genetic background.

In addition, striatal dopamine can potentially stimulate subventricular zone proliferation, so L-DOPA treatment in patients with Parkinson's disease could potentially rescue any effect of dopaminergic denervation. Therefore, we included cases with incidental Lewy body disease, which have α-synuclein pathology, but did not receive L-DOPA treatment. In these cases we observed a similar variability in proliferation, excluding L-DOPA use as a major influence on proliferation and neural stem cells in our Parkinson's disease group. A possible confounding factor here may be age, since the cases with incidental Lewy body disease had a higher mean age. However, we did not find an overall correlation between proliferation and age, making it unlikely that our results in the cases with incidental Lewy body disease were influenced by their age. Taking all data together, there is significant variation in subventricular zone proliferation or neural stem cell number between individuals, which cannot be accounted for by a single factor, like Parkinson's disease pathology or use of medication. Patients with Parkinson's disease have, on average, an equal level of proliferation, with a similar variability as controls.

Also the number of neural stem cells, as measured by GFAP-δ expression, is highly variable and not significantly different between controls and Parkinson's disease donors in our data set. GFAP-δ expression in the striatal subventricular zone is generally lower than in other areas we have studied before (van den Berge *et al*., 2010), which is also seen for pan-GFAP expression (Quinones-Hinojosa *et al*., 2006). Although the number of stem

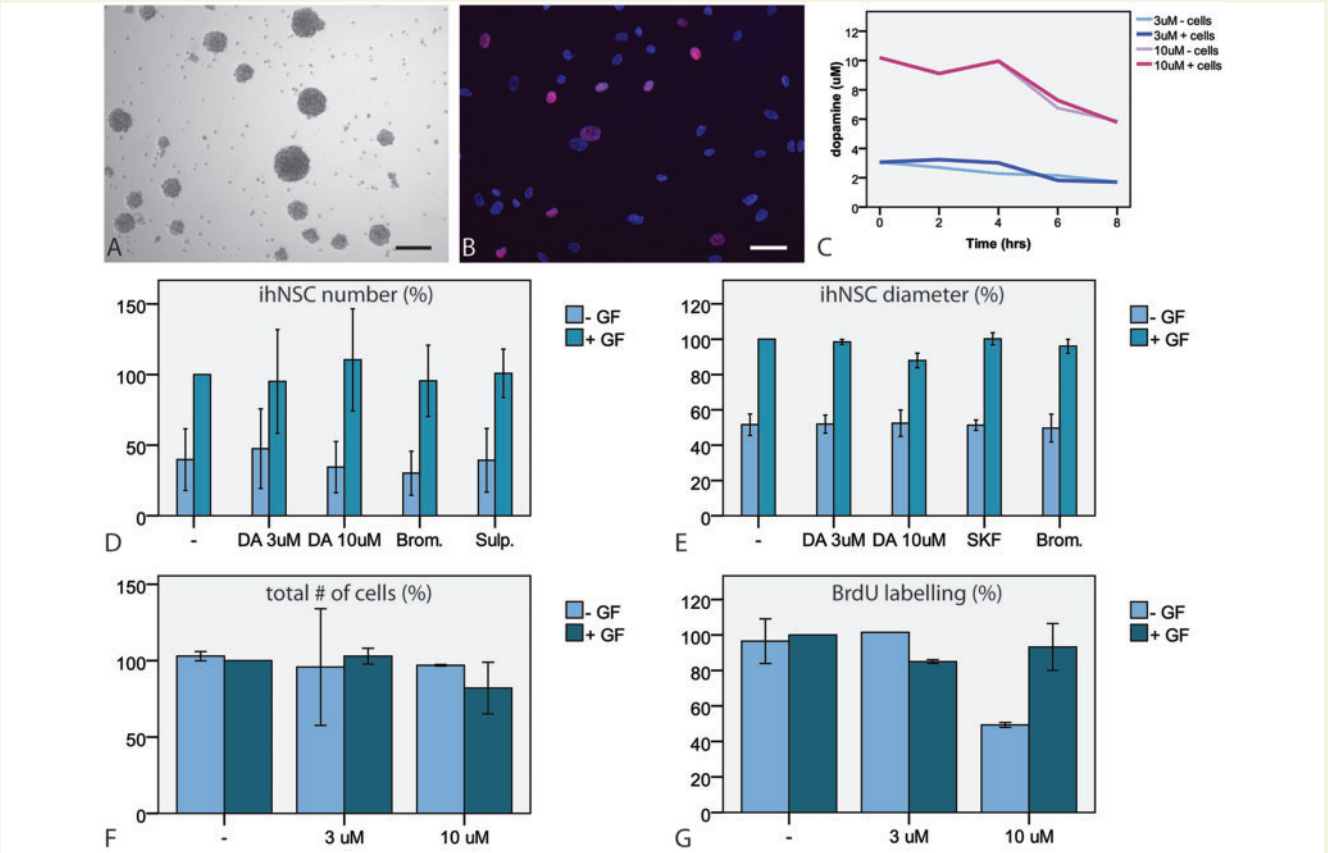

Figure 6 The effect of dopamine on proliferation of human neural precursor lines. (**A**) Phase contrast image of immortalized human neural stem cells (ihNSCs) in culture, forming neurospheres. (**B**) CB660 cells (nuclei in *blue*) stained for bromodeoxyuridine (BrdU) incorporation (*red*). (**C**) Measurement of dopamine stability in medium with and without cells. (**D**, **E**) Average number (**D**) and diameter (**E**) of neurospheres after treatment with dopamine or dopamine agonists in the absence or presence of growth factors (GF). The condition with growth factors without agonists was set to 100% to normalize data between experiments. (**F**) Average number of CB660 cells after treatment with dopamine in the absence or presence of growth factors. The condition with growth factors without dopamine was set to 100%. (**G**) Percentage of bromodeoxyuridine-positive CB660 cells after treatment with dopamine in the absence or presence of growth factors. The condition with growth factors without dopamine was set to 100%. Blue staining in (**B**) is Hoechst staining for cell nuclei; Bromo = D2 receptor agonist bromocryptine; DA = dopamine; SKF = D1 receptor agonist SKF-38393; Sulp = D2 receptor antagonist sulpiride; scale bars indicate 250 μM in (**A**) and 50 μM in (**B**); graphs represent mean ± SEM.

cells seems to be less in this area, we can still detect neural stem cells and isolate them, also in the Parkinson's disease donors. This finding is supported by our ability to culture multipotent neurospheres from the Parkinson's disease brain, with a success rate similar to that in control donors. GFAP-δ-immunoreactivity in the olfactory bulb was unchanged in Parkinson's disease donors compared with controls suggesting that the number of putative precursor cells in the olfactory bulb is not affected by Parkinson's disease pathology.

To study the effect of dopamine depletion on the mouse subventricular zone, we employed a chronic MPTP model, which results in a long-lasting degeneration of the dopaminergic neurons in the substantia nigra and denervation of the striatum (Petroske *et al.*, 2001). In addition, α-synuclein-positive inclusions have been observed in the substantia nigra and cortex in this model (Meredith *et al.*, 2002). Currently, this is the only established model that combines these characteristics, which is an important improvement over acute and subacute MPTP models (Meredith *et al.*, 2008). Especially the chronic loss of striatal dopaminergic innervations is unique to this model, and is more representative of the chronic nature of the human Parkinson's disease pathology. The discrepancy between published data and our current data on PCNA expression might be caused by this difference between the mouse models, but it is in concurrence with our data on the human subventricular zone. We saw a slight increase in pHH3 expression, which is consistent with data in other rodent Parkinson's disease models, such as the 6-hydroxydopamine model (Liu *et al.*, 2006; Aponso *et al.*, 2008). However, the number of pHH3 cells was very low in both control and MPTP-treated mice, making it questionable if this increase will have physiological relevance. In general, data on the subventricular zone in Parkinson's disease models are mixed, with studies reporting an increase (Liu *et al.*, 2006; Aponso *et al.*, 2008), decrease (Baker *et al.*, 2004; Höglinger *et al.*, 2004; Winner *et al.*,

2006, 2008*b*) or no change (Winner *et al.*, 2004; Marxreiter *et al.*, 2009) in subventricular zone proliferation. We did not use bromodeoxyuridine labelling in the mouse subventricular zone, but it has been shown that PCNA expression correlates with bromodeoxyuridine labelling (Mokry *et al.*, 2003). However, there are some drawbacks to using PCNA as a proliferation marker, such as its long half-life and ability to label apoptotic cells (Eisch and Mandyam, 2007), therefore we included pHH3, which is very specifically expressed in mitotic cells (Juan *et al.*, 1998).

We also investigated the effect of dopamine on human neural stem cells in culture. For this purpose, we added dopamine to two different foetal neural stem cell lines. This addition did not change the proliferation rate of these cell lines as measured by neurosphere number and diameter or bromodeoxyuridine incorporation. To one of the lines, we also added dopamine receptor agonists, which again did not have an effect on proliferation of the neural stem cell line. From these experiments, we can conclude that dopaminergic stimulation is not important for human neural stem cells proliferation. Previous studies with cultured neural precursor cells did show an effect of dopamine on these cells (Coronas *et al.*, 2004; Höglinger *et al.*, 2004; Kippin *et al.*, 2005), but these experiments were done only in rodent cultures. This implies that there may be a species difference in the proliferative reaction of neural precursor cells to dopamine. We did not yet examine differentiation of the neural stem cell lines, which may be affected by dopamine signalling.

The main finding of our study is that the striatal subventricular zone of patients with Parkinson's disease contains actively proliferating neural stem cells, and that the numbers and the proliferative capacity of this population are not affected by the disease in the groups of controls, cases with incidental Lewy body disease and cases with Parkinson's disease we studied. This means that future therapeutic interventions could be targeted to endogenous neural stem cells. This might be an attractive alternative for transplantation studies, since they pose several medical and ethical problems (as reviewed in Meyer *et al.*, 2010). These problems include limited efficacy, adverse side effects and ethical issues concerning the origin of the transplanted cells. Progress is being made towards stimulating endogenous neural stem cells to form dopaminergic neurons in the striatum (reviewed in Geraerts *et al.*, 2007), to rescue the existing dopamine deficit. Small steps towards this goal are being made using administration of different exogenous factors in Parkinson's disease animal models. Thus far, many studies show an increase in proliferation and migration of subventricular zone precursors (Cooper and Isacson, 2004; de Chevigny *et al.*, 2008; Peng *et al.*, 2008; Winner *et al.*, 2008*a*, 2009), but only a few studies have shown neuronal differentiation (Fallon *et al.*, 2000; Mohapel *et al.*, 2005; Gonzalo-Gobernado *et al.*, 2009) or dopaminergic differentiation (Arias-Carrion *et al.*, 2004, 2006). An insight into neuronal repair by endogenous neural stem cells can be obtained from stroke models. After focal ischaemia, subventricular zone precursors increase proliferation and migration, and also neuronal differentiation in the damaged area (reviewed in Kernie and Parent, 2010). The molecular factors involved in this process are being elucidated, and might be of use to stimulate differentiation and survival in Parkinson's disease models.

In conclusion, our data show that the proliferation capacity and neural stem cell compartment in the subventricular zone are highly variable, but maintained in the untreated incidental Lewy body disease and L-DOPA-treated Parkinson's disease brain, which is in contrast to earlier studies. We also showed that viable neural stem cells can be isolated from brains of control subjects and patients with Parkinson's disease, corroborating our immunohistochemical data on the presence of neural stem cells in the subventricular zone. These findings provide a window of opportunity for therapeutic intervention, based on recruitment of endogenous neural stem cells.

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Supplementary material

Supplementary material is available at *Brain* online.

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