Growth factors regulate the survival and fate of cells derived from human neurospheres

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Cells isolated from the embryonic, neonatal, and adult rodent central nervous system divide in response to epidermal growth factor (EGF) and fibroblast growth factor 2 (FGF-2), while retaining the ability to differentiate into neurons and glia[1,2]. These cultures can be grown in aggregates termed neurospheres, which contain a heterogeneous mix of both multipotent stem cells and more restricted progenitor populations[3,4]. Neurospheres can also be generated from the embryonic human brain[5–7] and in some cases have been expanded for extended periods of time in culture[8–10]. However, the mechanisms controlling the number of neurons generated from human neurospheres are poorly understood. Here we show that maintaining cell–cell contact during the differentiation stage, in combination with growth factor administration, can increase the number of neurons generated under serum-free conditions from 8% to >60%. Neurotrophic factors 3 and 4 (NT3, NT4) and platelet-derived growth factor (PDGF) were the most potent, and acted by increasing neuronal survival rather than inducing neuronal phenotype. Following differentiation, the neurons could survive dissociation and either replating or transplantation into the adult rat brain. This experimental system provides a practically limitless supply of enriched, non-genetically transformed neurons. These should be useful for both neuroactive drug screening *in vitro* and possibly cell therapy for neurodegenerative diseases.

Crucial to the success of providing a reliable source of human nervous tissue through neural precursor cultures is tight control of the growth and differentiation media. We have reported elsewhere that FGF-2 can promote the long-term growth of human neurospheres, providing that cell–cell contact is maintained using a unique "chopping" method for passaging[8]. Interestingly, this same method cannot prevent the lack of growth seen after only a few weeks of rodent neurosphere expansion[11]. Furthermore, we observed that human neurospheres treated with FGF-2 were prone to attachment at later passages but that EGF-responsive neurospheres remained in suspension[8]. Subsequent reports have confirmed that human FGF-2-responsive neurospheres cannot be grown for extended periods of time as neurospheres, but this may be overcome by the addition of leukemia inhibitory factor (LIF) to the medium[9]. This factor may act to prevent the differentiation and attachment of the cells at passaging. Long-term neurosphere cultures have also been generated using a combination of EGF and FGF-2 in the medium[10]. Future refinements of the medium or passaging technique should improve the

growth characteristics of these cells. However, control of their subsequent differentiation has not been as extensively studied.

In the present report, cultures were initiated from the cortex of eight-week-old post-mortem human brain tissue in a combination of EGF and FGF-2 for four weeks. The neurospheres were then transferred to medium with EGF alone and could be expanded using the chopping method[8] for up to 300 days, after which division rates slowed, possibly as a consequence of lack of telomerase expression and erosion of telomeres[12]. We first wanted to see if the numbers of neurons and glia generated by these EGF-responsive neurospheres could be modulated by growth factors. The neurotrophin family includes nerve growth factor (NGF), brain-derived growth factor (BDNF), NT3, and NT4, which have a broad range of effects on neuronal survival and maturation. These factors act by stimulating the TRK family of receptor tyrosine kinases (for review see ref. 13). Neurospheres were triturated to a single-cell suspension and plated onto poly-L-lysine-coated glass coverslips (Fig. 1A). After 24 h they were stained with an antibody directed against TrkC and known to cross-react with TrkA and TrkB. A subpopulation of cells with neuronal morphology expressed Trk receptors (Fig. 1B,C), indicating that they may be responsive to neurotrophins. In addition, 10 min exposure to neurotrophins phosphorylated the Trk receptor in sister cultures (data not shown).

There are many other growth factor families with known effects on neural tissues. Ciliary neurotrophic factor (CNTF) enhances neuronal survival in primary rodent hippocampal cultures[14] and can also induce astrocyte differentiation from hippocampal progenitors[15]. Platelet-derived growth factor (PDGF) is expressed widely in the developing brain[16], and has been shown to increase the number of neurons in both primary and progenitor cell cultures[15,17]. Following neurosphere dissociation and plating in the absence of growth factors or serum, ~8% of the cells differentiated into neurons and 50% into astrocytes after seven days (Fig. 1D,E). The number of neurons could be increased to 15 and 18% by the addition of NT3 and NT4, respectively, at the time of plating (Fig. 1D). Interestingly, NT3 and NT4 decreased the number of astrocytes in these cultures to 40 and 35%, respectively (Fig. 1E). No significant effects on either neuronal or glial cell number were observed with BDNF, CNTF, and PDGF (Fig. 1D,E).

We have shown elsewhere that plating intact neurospheres on poly-L-lysine for 14 days in the presence of 1% fetal calf serum for the first 24 h resulted in the generation of up to 50% neurons in the region around the sphere[8], significantly higher than that seen with dissociated cultures. To assess which of these factors is most important, we wanted to develop a method of plating intact EGF-responsive spheres under serum-free conditions. However, in the absence of serum, spheres did not attach to poly-L-lysine-coated coverslips (data not shown). A variety of substrates including merosin, fibronectin, and laminin were tested for their ability to allow sphere attachment. Laminin provided the best adherence in serum-free conditions and was used throughout this study. We then developed a method for differentiating the cultures (Fig 1F): In stage I, the spheres were plated on poly-L-lysine/laminin, leading to the formation of radial processes that stained for radial glial markers and to the migration of neuroblasts; in stage II, the cultures were trypsinized, dissociated, and replated as a monolayer culture. Because both the remaining spheres and the cells that have migrated are collected in stage II, this method reveals the total numbers of neurons and astrocytes generated by these cultures. This technique

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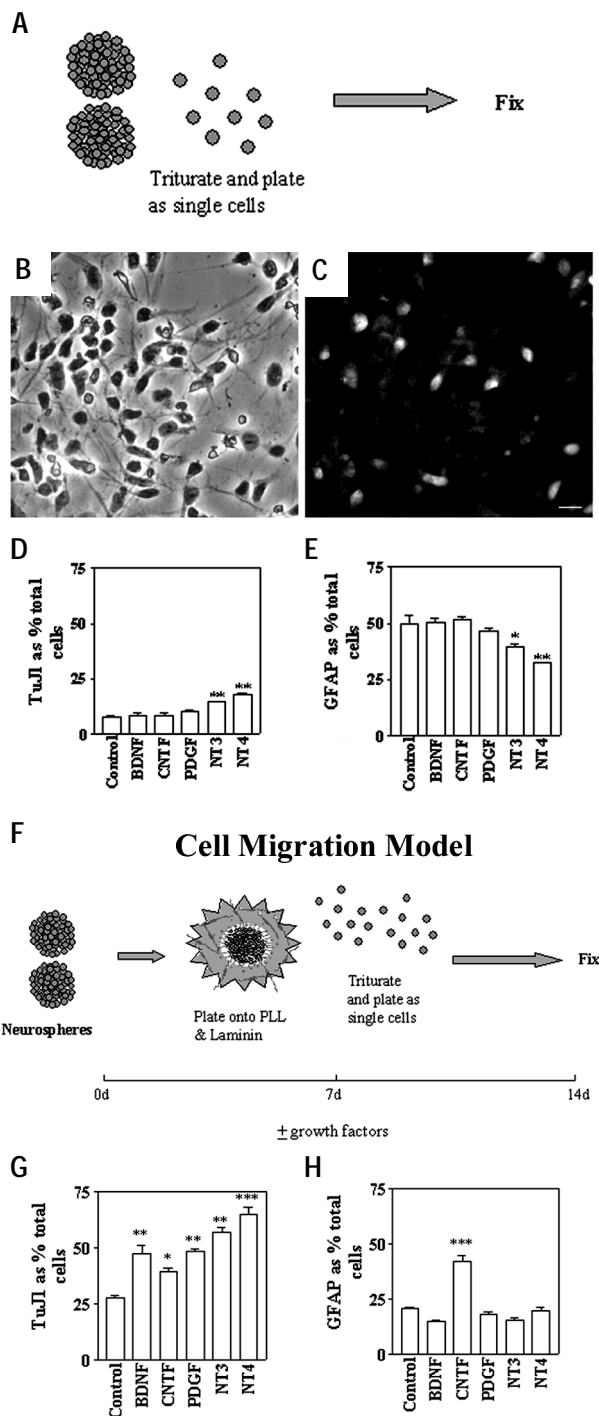
Figure 1. Controlling the fate of human neurospheres. (A) Neurospheres were dissociated and plated for seven days. (B) Cells derived from neurospheres express Trk receptors, detected using an antibody to TrkC that also reacts with TrkB. Photomicrographs represent (B) phase and (C) the same field demonstrating Trk immunoreactivity 24 h after plating. Sister cultures were allowed to differentiate for seven days in the presence of growth factors. NT3 and NT4 (D) significantly increased the number of neurons and (E) significantly decreased the number of astrocytes in cultures when neurospheres were dissociated and plated for seven days. *$P < 0.05$; **$P < 0.01$ versus control. (F) Cell migration model. Stage I (days 1–7): Neurospheres were plated as whole spheres on poly-L-lysine/laminin-coated T25 flasks in the presence or absence of one of BDNF, NT3, NT4, CNTF or PDGF-$\beta\beta$. The concentration of all growth factors used was 20 ng/ml. Stage II (days 7–14): Cells were subsequently triturated into a single-cell suspension and replated onto poly-L-lysine-coated coverslips in the presence or absence of one of the above-named growth factors. (G) All growth factors tested increased the number of neurons over control values when the cell migration model was used, whereas (H) CNTF significantly increased the number of astrocytes. These experiments were carried out using two different human samples, which yielded similar results. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ versus control. Scale bar represents 30 μm.

(which was consistently <0.05%, data not shown), or changed the number of glutamate-immunopositive neurons, which was ~40%. Interestingly, CNTF, but not the other factors, increased the proportion of γ-aminobutyric acid (GABA)-immunopositive neurons from 30% to 70% ($P < 0.01$). We next extended these studies with CNTF and NT4 in cortical samples from another three embryos. These data showed a similar pattern to that seen before. Although there was a trend, the total number of surviving cells did not differ significantly among the groups (Fig. 2A–C).

The combination of plating whole spheres and growth factor treatment efficiently generated neurons. What is the mechanism underlying this effect? Time-lapse observation of the spheres following plating revealed that radiating processes appeared within the first few hours, pushing out onto the laminin and forming around the plated sphere a ring of fibers that were positive for radial glia markers and nestin (Fig. 2D). TuJ1-positive cells with long leading growth processes then migrated along the radial fibers and onto the laminin (Fig. 2D). Eventually, the entire coverslip became populated with neuronal and glial cells, which formed a dense tissue mat. There were numerous examples of BrdU/TuJ1+ neurons when cultures were pulsed with 5-bromodeoxyuridine (BrdU) for 24 h. How do these newly generated cells respond to neurotrophin treatment? Extracellular signal-regulated kinase (ERK) is known to stimulate neurite outgrowth and neurotrophin responses (for review see ref. 18), whereas Akt/PKB has been shown to promote growth factor–mediated cell survival and to block apoptosis[19]. Following 10 min stimulation, BDNF and NT4 induced a large amount of ERK expression; PDGF and NT3 induced a smaller amount, whereas CNTF had no effect on basal levels (Fig 2E). BDNF and NT4 induced the highest P-AKT expression levels; PDGF and NT3 had a stronger response than that obtained for ERK; and CNTF elicited no response (Fig. 2E). Together, these data suggest that the neurotrophins, but not CNTF, were able to activate pathways that blocked apoptosis. To investigate this further, we dissociated and plated neurospheres without growth factors for seven days (Fig. 1A), at which time ~50% of the cells were positive for terminal deoxynucleotidyl transferase-mediated dUTP-X 3′ nick end labeling (TUNEL) (Fig. 2F). Under these conditions, NT4 reduced the number of TUNEL-positive cells to ~35%, whereas CNTF had no significant effect. A similar result was seen using the cell migration model (Fig. 2F,G).

Recent studies using similar human neural precursor cells grown in both EGF and FGF-2 have shown that neurons derived from these cultures are electrophysiologically active[20], and in some cases generate action potentials[10]. Here we used whole-cell patch-clamp techniques to study the effects of several neurotransmitters on putative

alone, in the absence of growth factors or serum, increased the percentage of neurons from 8% in dissociated cultures to ~26% (figs 1G, 2A). All growth factors tested increased the number of neurons generated in this assay, but the greatest effect was seen in NT4-treated cultures, where >60% of the cells were neuronal (figs 1G, 2B). Plating of intact spheres to allow migration also significantly decreased the numbers of astrocytes in these cultures from approximately 55 to 20% (Fig. 1H). CNTF was the only growth factor capable of increasing the number of astrocytes to 42% compared to the 20% found in control cultures (Fig. 1H). All growth factors except NT3 increased cell-body size from ~52 μm^2 to ~66 μm^2 ($P < 0.05$). None of the factors induced tyrosine hydroxylase (TH) expression
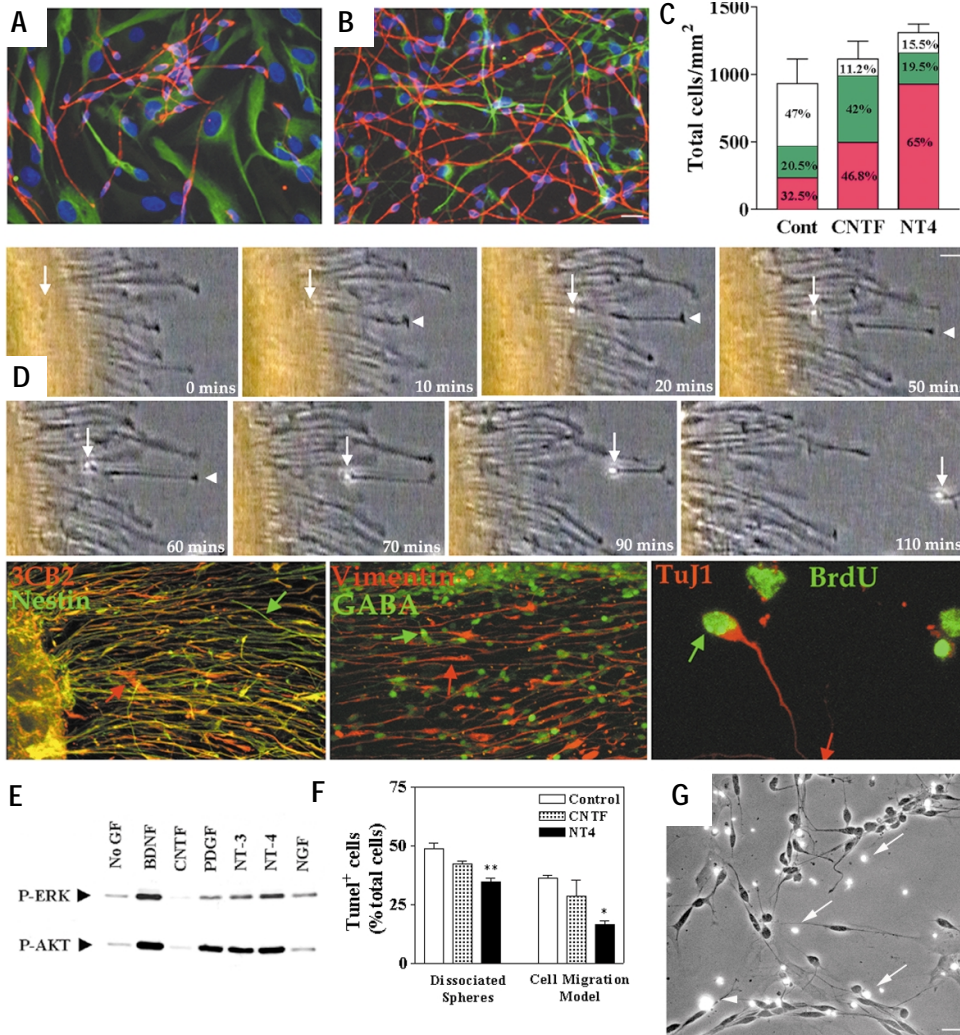
Figure 2. Mechanisms of neuronal generation and survival. Photomicrographs represent the TuJ1+ neurons (red), GFAP+ astrocytes (green) and Hoescht-labeled nuclei (blue), generated (A) in the absence of any growth factor (B) after treatment with NT4. The total cell numbers per field are shown in (C). The red part of each bar represents the percentage of cells that differentiated into TuJ1+ neurons, the green the percentage of cells that differentiated into GFAP+ astrocytes, and the white the unlabeled part of the population. $n = 5$ different human samples. **$P < 0.001$ versus control. (D) Time-lapse microscopy. One sphere plated onto poly-L-lysine/laminin for 5 h before filming is shown. 3CB2, nestin, and vimentin+ fibers are seen migrating from the sphere. Neurons stain positive for GABA, TuJ1, and BrdU. (E) The neurotrophins and PDGF are capable of phosphorylating ERK (pERK) and Akt (pAKT) in human neurospheres. (F) The number of TUNEL+ cells after seven days plating is not affected by CNTF, but is significantly reduced by NT4 when cells are dissociated and plated (left hand side of graph) and when the cell migration model is used (right hand side of graph). $n = 3$ different human samples. (G) Photomicrograph represents a phase image overlaid with the same TUNEL+ image following the cell migration model/protocol. Arrows indicate cells that are already dead, and arrowheads indicate a cell that is undergoing cell death. *$P < 0.05$, **$P < 0.01$ versus control. Scale bar represents 20 μm.

neurons. Results were compared to primary cultured cortical neurons from eight-week-old human cortex. After three weeks in culture, cells exhibiting neuronal morphology (Fig. 3A) showed responses to GABA, kainate, and N-methyl-D-aspartate (NMDA; Fig. 3B). Whereas the GABA responses were significantly smaller than in primary cultures (69 ± 29 pA ($n = 10$) versus $5,432 \pm 770$ pA ($n = 7$)), the kainate responses were of similar magnitude (100–200 pA). Small currents were observed for NMDA (5–40 pA), compared with 111 pA in primary cultures. Neurotransmitter responses were not significantly affected by treatment with BDNF, NT-3, or NT-4. To look for other physiological characteristics of neurons, we performed a GABA uptake assay. Control cultures demonstrated GABA uptake, and uptake increased after NT-4 treatment, reflecting the increased numbers of neurons (Fig. 3C). Finally, either whole neurospheres or cultures pre-differentiated in NT4 for seven days (Fig. 1F) were trypsinized and transplanted into 6-OHDA-lesioned rat striatum. In these preliminary studies, cells transplanted directly from neurospheres (dissociated into single cells) displayed thinner grafts, and large numbers of cells were found to have migrated away from the core (Fig. 3D). Cells pre-differentiated in NT4 for seven days formed larger distinct masses with little evidence of cell migration away from the graft (Fig. 3E).

Increasing the number of neurons generated from human neurospheres is of interest to basic scientists, drug companies, and cell therapy programs. We have shown that plating intact neurospheres in the presence of growth factors significantly increases the number of developing neurons. This method avoids the mechanical trauma associated with dissociation, and may improve neuronal survival for this reason. Plating intact spheres rather than dissociated cells also allows precursor cells a window of time to establish their fate in a three-dimensional environment while maintaining communication with neighboring cells and migrating along radial glia. Although NT3 and NT4 had some effect on the number of neurons in dissociated cultures, all factors tested increased neuronal numbers when the cell migration model was used. Thus, neurotrophic factors appear more likely to affect the differentiation and survival of neural progenitors when they are undergoing migration from intact spheres. This situation might approximate better the environment in which neuronal progenitors develop in vivo[21].

Neurotrophins that affect neuronal differentiation may act by protecting newly generated neuroblasts migrating from intact spheres. In contrast, CNTF seems to direct the fate of noncommitted precursors toward the astrocyte lineage. Somewhat surprisingly, CNTF also increased the number of differentiated neurons expressing a GABA-ergic phenotype, over and above its modest effects on total neuronal number. Other workers have shown that CNTF protects GABA neurons from degeneration in a number of models[14,22,23], but our results suggest that it may also be able to upregulate GABA in existing neurons. This factor should therefore be useful for generating highly enriched cultures of GABA-positive neurons from human
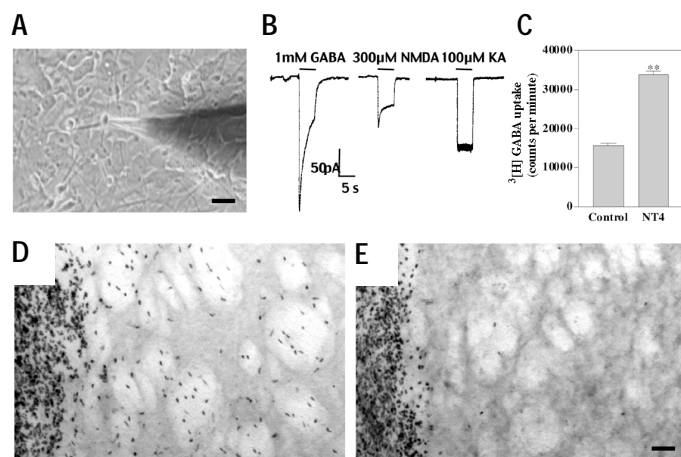
Figure 3. Electrophysiological responses and transplantation of cultured neurons. (A) Phase-contrast photograph illustrating patch formation on cells with neuronal morphology. (B) Electrophysiological recordings of responses to 1 mM GABA, 300 μM *N*-methyl-D-aspartate, and 100 μM kainate, from cultured cells derived from human neurospheres, demonstrating the presence of GABA-A receptors, NMDA receptors, and kainate receptors on these cells. Cell membranes were voltage-clamped at –60 mV, and application times are illustrated by the bar above each trace. (C) [³H]GABA uptake. NT4 significantly increased GABA uptake over control conditions. **$P < 0.01$ versus control. Staining of neurosphere transplants (D) or cells treated according to the cell migration model (E) with an antibody directed against a human nuclear marker. Pre-differentiating the cells in vitro leads to far less migration away from the transplant site and resembles a primary neuronal transplant. Scale bar represents 40 μm for (A) and 100 μm for (D) and (E).

neurospheres. In the only other report of growth factor effects on human neurospheres, Galli *et al.* showed that PDGF decreased the number of neurons that developed from dissociated neurosphere cultures[24]. This decrease in neurons was not at the expense of astrocytes, because they found no astrocytes in PDGF-treated cultures. In contrast, we found that PDGF had no effect under identical conditions (plating dissociated spheres). However, in the migration model, PDGF significantly increased the number of new neurons. This agrees with data from rodent cultures, where PDGF plays an instructive role in promoting neuronal fate in E14 cortical progenitor cells[17,25] or expanded populations of hippocampal progenitors[15].

In light of the positive effects seen following transplantation of fetal dopamine neurons into patients with Parkinson's disease[26], there is great interest in generating dopamine neurons from human neurospheres. Furthermore, because dyskinesias are seen in some transplanted patients[27], it is crucial to know exactly which types of cell are grafted. Although FGF-2–responsive human neurospheres derived from the cortex can survive transplantation into a rat model of Parkinson's disease (PD), and extend many axons into the host brain, very few dopamine neurons can be found in these transplants[12]. Neural precursors isolated directly from the developing rodent mesencephalon can proliferate a few times and still generate dopamine neurons[28], but not following longer periods of expansion. Rodent neurospheres or neural cell lines express dopamine markers when specific transcription factors are overexpressed[29,30], or following administration of complex cocktails of growth factors and cytokines[31]. However, human cells appear to be more difficult to induce using conventional plating methods, giving rise only to very small numbers of dopamine neurons under the same conditions (unpublished observations; ref. 9). The overexpression of transcription factors in combination with plating whole spheres and neurotrophins as described here may yield larger numbers of human dopamine neurons for both drug discovery and transplantation in

PD. Other neurodegenerative diseases such as stroke and Huntington's disease may be amenable to cell therapy techniques that require human neurons that are not dopaminergic[32]. Our technique for generating highly enriched human neuronal cultures should be useful for both these types of clinical studies, and for general programs of drug discovery and neurobiological research.

Experimental protocol

Human fetal tissue (8–12 weeks postconception) was used in this study. The methods of collection conform to the arrangements set out by the Department of Health in the United Kingdom. Precursor cells derived from the cortex of human fetal brain were grown as free-floating aggregates (neurospheres), and optimal expansion was achieved using methods described elsewhere[8]. Cells were grown in EGF + FGF-2 for the first four weeks, then switched to EGF alone for the remaining weeks. At the end of 15–23 weeks, expansion neurospheres were dissociated using trypsin (0.1%), adjusted to 1,000 cells/μl and plated in a 30 μl drop onto a poly-L-lysine/laminin-coated coverslip (Fig 1A) in B27 medium supplemented with one of BDNF, PDGF-ββ, NT3, NT4 (all at a concentration of 20 ng/ml; R & D Systems, Minneapolis, MN) or CNTF (20 ng/ml; TCS Biologicals, Buckingham, UK). After 24 h the cells were stained for TrkC, or at seven days for TuJ1 and GFAP. Alternatively, a method in which intact spheres were plated was used, as described in the Results section (Fig. 1F). In some cases ³[H]GABA uptake was assessed as described[33].

TUNEL assay. Cells were prepared as described above under three conditions: control, NT4, and CNTF. Staining was carried out on cells seven days post plating according to manufacturer's instructions (Boehringer Mannheim).

Preparation of cells for analysis of mitogen-activated protein (MAP) kinase and phosphatidylinositol 3 (PI-3) kinase pathways. Whole spheres were plated into T25 flasks coated with poly-L-lysine/laminin. At seven days, cells were stimulated for 10 min using 100 ng/ml of each growth factor, and were then lysed in 200 μl of New England Biolabs (NEB) lysis buffer and western blotted. The resulting membranes were probed with either α-phospho-Trk 490 (1:500; New England BioLabs, Beverly, MA), α-phospho-Akt (Ser473) (1:2,000; Upstate Biotechnology, Lake Placid, NY), or α-phospho ERK (Thr202/Tyr204) (1:500; Santa Cruz, CA) overnight at 4°C. The phosphorylated bands were visualized with α-rabbit (for Trk and Akt) and α-mouse (for ERK) conjugated to horseradish peroxidase (HRP).

Immunohistochemistry. Cells were fixed in 4% paraformaldehyde in PBS containing 4% sucrose for 20 min. Immunocytochemistry was carried out using standard protocols. For BrdU staining, cells were treated with 2 M HCl for 20 min at 37°C followed by 2 × 5 min washes in 0.1 M sodium borate buffer. 3% normal goat serum was used to block all cells before addition of primary antibodies. Primary antibodies and dilutions were as follows: β-tubulin type III monoclonal (TuJ1, 1:500; Sigma, St. Louis, MO), GFAP polyclonal (1:1,000; DAKO, Glostrup, Denmark), GFAP monoclonal (1:500; Chemicon, Harrow, UK), TH monoclonal (1:500; Chemicon), Vimentin monoclonal (1:500; DAKO), BrdU monoclonal (1:300; Roche, Sussex, UK), 3CB2 monoclonal (1:500; Developmental Studies Hybridoma Bank, Univ. of Iowa, Iowa City), TrkC polyclonal (1:500; Santa Cruz), GABA polyclonal (1:250; Sigma), Glutamate polyclonal (1:5,000; Sigma), human nuclear (1:500; Chemicon). Cells were then reacted with appropriate secondary antibodies (Harlan Seralab, Harlan, UK) conjugated to either biotin or fluorescein isothiocyanate (FITC) for 1 h at room temperature. Biotin-conjugated antibodies were then visualized using streptavidin–Rhodamine (1:200). Hoescht nuclear stain (1:5,000) was included in the final antibody applications. The size of the neuronal soma of TuJ1+ neurons was measured by drawing around the cell body of the neurons using a digitized image analysis system.

Electrophysiology. Glass coverslips containing a monolayer of cells were placed in a chamber on the stage of a Nikon Diaphot inverted microscope. Cells were perfused continuously with artificial cerebral spinal fluid (aCSF) containing (in mM): 149 NaCl, 3.25 KCl, 2 CaCl₂, 2 MgCl₂, 10 HEPES, 11 D-glucose, D(+)-sucrose, pH 7.4, and observed with phase-contrast optics. Fire-polished patch pipettes were pulled on a WZ, DMZ-Universal puller

(Zeiss, Augsburg, Germany) using conventional 120TF-10 electrode glass. Pipette tip diameter was ~1.5–2.5 μm, with resistances ~4 MΩ. The intracellular solution contained (in mM): 130 CsCl, 10 HEPES, 10 BAPTA.Cs, 5 ATP.Mg, 0.1 Leupeptin, 1 MgCl$_2$, 100 μM NaVO$_3$, pH adjusted to 7.3 with CsOH and 320–340 mOsm. Cells were voltage-clamped at –60 mV using an Axon 200B amplifier (Axon Instruments, Foster City, CA). Drug solutions were applied to the cells using a multibarrel drug delivery system, which pivots the barrels into place using a stepping motor. This ensured rapid application and washout of the drug. Drugs were applied to the cell for 5 s with a 30 s washout period between applications.

Transplantation. Nude rats (strain RH/RNU) received unilateral 6-hydroxydopamine (6-OHDA) lesions as described elsewhere[12]. Seven days before transplantation surgery, spheres were plated as described in Figure 1F in the presence of NT4. On the day of surgery these cells were dissociated with trypsin and DNase, and then grafted at a density of 500,000 cells per rat using coordinates described elsewhere[12]. A second group of rats received sister spheres, which were treated with trypsin and DNase but were not predifferentiated before transplantation. Animals were killed 12 weeks after transplantation as described[12].

Cell counts and statistics. In order to determine the number of neurons and astrocytes, photographs of immunostained cells were taken using a high-resolution digital camera (Nikon). Five fields per 13 mm coverslip were counted with five coverslips per condition in each experiment. Statistical comparisons were made by ANOVA with post hoc Newman–Keuls test when more than two groups were involved. Data were expressed as mean ± s.e.m.

1. Gage, F.H. Mammalian neural stem cells. *Science* **287**, 443–448 (2000).
2. McKay, R.D.G. Stem cells in the central nervous system. *Science* **276**, 66–71 (1997).
3. Reynolds, B.A. & Weiss, S. Clonal and population analyses demonstrate that an EGF-responsive mammalian embryonic CNS precursor is a stem cell. *Dev. Biol.* **175**, 1–13 (1996).
4. Svendsen, C.N. & Caldwell, M.A. Neural stem cells in the developing central nervous system: implications for cell therapy through transplantation. *Prog. Brain Res.* **127**, 13–24 (2000).
5. Flax, J.D. *et al.* Engraftable human neural stem cells respond to developmental cues, replace neurons, and express foreign antigens. *Nat. Biotechnol.* **16**, 1033–1039 (1998).
6. Quinn, S.M. *et al.* Lineage restriction of neuroepithelial precursor cells from fetal human spinal cord. *J. Neurosci. Res.* **57**, 590–602 (1999).
7. Svendsen, C.N. *et al.* Survival and differentiation of rat and human epidermal growth factor-responsive precursor cells following grafting into the lesioned adult central nervous system. *Exp. Neurol.* **137**, 376–388 (1996).
8. Svendsen, C.N. *et al.* A new method for the rapid and long term growth of human neural precursor cells. *J. Neurosci. Methods* **85**, 141–152 (1998).
9. Carpenter, M.K. *et al.* In vitro expansion of a multipotent population of human neural progenitor cells. *Exp. Neurol.* **158**, 265–278 (1999).
10. Vescovi, A.L. *et al.* Isolation and cloning of multipotential stem cells from the embryonic human CNS and establishment of transplantable human neural stem cell lines by epigenetic stimulation. *Exp. Neurol.* **156**, 71–83 (1999).
11. Svendsen, C.N. *et al.* Restricted growth potential of rat neural precursors as compared to mouse. *Dev. Brain Res.* **99**, 253–258 (1997).
12. Ostenfeld, T. *et al.* Human neural precursor cells express low levels of telomerase in vitro and show diminishing cell proliferation with extensive axonal outgrowth following transplantation. *Exp. Neurol.* **164**, 215–226 (2000).
13. Hefti, F. Pharmacology of neurotrophic factors. *Annu. Rev. Pharmacol. Toxicol.* **37**, 239–267 (1997).
14. Ip, N.Y. *et al.* Ciliary neurotrophic factor enhances neuronal survival in embryonic rat hippocampal culture. *J. Neurosci.* **11**, 3124–3134 (1998).
15. Johe, K.K. *et al.* Single factors direct the differentiation of stem cells from the fetal and adult central nervous system. *Genes Devel.* **10**, 3129–3140 (1996).
16. Yeh, H.J. *et al.* PDGF A-chain gene is expressed by mammalian neurons during development and in maturity. *Cell* **64**, 209–216 (1991).
17. Williams, B.P. *et al.* A PDGF-regulated immediate early gene response initiates neuronal differentiation in ventricular zone progenitor cells. *Neuron* **18**, 553–562 (1997).
18. Greene, L.A. & Kaplan, D.R. Early events in neurotrophin signalling via Trk and p75 receptors. *Curr. Opin. Neurobiol.* **5**, 579–587 (1995).
19. Dudek, H. *et al.* Regulation of neuronal survival by the serine-threonine protein kinase Akt. *Science* **275**, 661–665 (1997).
20. Piper, D.R. *et al.* Immunocytochemical and physiological characterization of a population of cultured human neural precursors. *J. Neurophysiol.* **84**, 534–548 (2000).
21. Kornack, D.R. & Rakic, P. Radial and horizontal deployment of clonally related cells in the primate neocortex: relationship to distinct mitotic lineages. *Neuron* **15**, 311–321 (1995).
22. Emerich, D.F. *et al.* Implants of encapsulated human CNTF-producing fibroblasts prevent behavioural deficits and striatal degeneration in a rodent model of Huntington's disease. *J. Neurosci.* **16**, 5168–5181 (1996).
23. Larkfors, L. Lindsay, R.M. & Alderson, R.F. Characterization of the responses of Purkinje cells to neurotrophin treatment. *J. Neurochem.* **66**, 1362–1373 (1996).
24. Galli, R. *et al.* Regulation of neuronal differentiation in human CNS stem cell progeny by leukemia inhibitory factor. *Dev. Neurosci.* **22**, 86–95 (2000).
25. Bonni, A. *et al.* Regulation of gliogenesis in the central nervous system by JAK/STAT signalling pathway. *Science* **278**, 477–483 (1997).
26. Lindvall, O. Neural transplantation: a hope for patients with Parkinson's disease. *NeuroReport* **8**, iii–x (1997).
27. Freed, C.R. *et al.* Transplantaion of embryonic dopamine neurons for severe Parkinson's disease. *N. Engl. J. Med.* **344**, 710–719 (2001)
28. Studer, L., Tabar, V. & McKay, R.D. Transplantation of expanded mesencephalic precursors leads to recovery in parkinsonian rats. *Nat. Neurosci.* **1**, 290–295 (1998).
29. Wagner, J. *et al.* Induction of midbrain dopaminergic phenotype in *Nurr*1-overexpressing neural stem cells by type1 astrocytes. *Nat. Biotechnol.* **17**, 653–659 (1999).
30. Sakurada, K. *et al.* Nurr1, an orphan nuclear receptor, is a transcriptional activator of endogenous tyrosine hydroxylase in neural progenitor cells derived from the adult brain. *Development* **126**, 4017–4026 (1999).
31. Ling, Z.-D. *et al.* Differentiation of mesencephalic progenitor cells into dopaminergic neurons by cytokines. *Exp. Neurol.* **149**, 411–423 (1997).
32. Bjorklund, A. & Lindvall, O. Self-repair in the brain. *Nature* **405**, 892–895 (2000).
33. Ciesielski-Treska, J. *et al.* Chromogranin A induces a neurotoxic phenotype in brain microglial cells. *J. Biol. Chem.* **273**, 14339–14346 (1998).