OPINION

# Gene therapy: can neural stem cells deliver?

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Abstract | Neural stem cells are a self-renewing population that generates the neurons and glia of the developing brain. They can be isolated, proliferated, genetically manipulated and differentiated *in vitro* and reintroduced into a developing, adult or pathologically altered CNS. Neural stem cells have been considered for use in cell replacement therapies in various neurodegenerative diseases, and an unexpected and potentially valuable characteristic of these cells has recently been revealed — they are highly migratory and seem to be attracted to areas of brain pathology such as ischaemic and neoplastic lesions. Here, we speculate on the ways in which neural stem cells might be exploited as delivery vehicles for gene therapy in the CNS.

Neural stem cells can be defined operationally as cells that can continuously self-renew and have the potential to generate intermediate and mature cells of both glial and neuronal lineages[1]. There are various subpopulations of neural stem cells that could be restricted to particular developmental stages or regions of the mature brain, and each of these populations is expected to have specific biological features[2,3]. It remains unclear whether cultured cells that are derived from the nervous system and fit the operational definition of neural stem cells — multipotency and the ability to self-renew — are identical to resident populations that have been reported *in vivo*. In addition, as there are few consensus criteria that can be used to define neural stem cells *in vitro*, the cells known as neural stem cells in one laboratory may differ considerably from similarly named cells derived in another laboratory. For the purposes of this review, we use an inclusive view, assuming that cells that are called neural stem cells by individual investigators do have common features that allow generalization. However, we do add the caveat that all claims made for a particular neural stem cell line or preparation might not apply to all populations (for more detailed data on this broad topic see REFS 3–6; for reviews, see REFS 2,7,8).

## Neural stem cell homing and drug delivery

The migratory abilities of endogenous and exogenous neural stem cells are well known, and it has been speculated for many years that these properties, along with the cells' differentiative abilities, might be harnessed for replacing neurons in degenerative disease. In 2000, some reports showed for the first time how these cells might be used in a novel way, not for cell replacement, but to deliver therapeutic substances to specific sites in the brain[9–11]. These reports showed that neural stem cells that were transplanted into animal models of brain neoplasia were found near metastatic tumour cells far from the site of their transplantation[9–11] (FIGS 1,2). These observations suggest that neural stem cells engineered to have chemotherapeutic qualities might be used to track down and destroy malignant cells. This opens up a possible new realm for stem cell therapy. Rather than being viewed solely as restorative cell therapeutics, the cells could help to solve one of the most persistent challenges of gene therapy — how to target therapeutic genes to diseased tissues.

The characteristics of neural stem cells make them suitable as therapeutic delivery vehicles for CNS disorders and distinguish them from other cell types that might be considered for this purpose. Unlike fibroblast cells, which might be suitable carriers in other organs, neural stem cells have the potential to integrate seamlessly into the host brain without disrupting normal function. For example, neural stem cells could differentiate into glia or neurons, but are unlikely to become connective tissue. In addition, neural stem cells can be propagated for long periods, and are therefore amenable to the techniques required for genetic manipulation. Because stem cells can disperse throughout the brain after transplantation, the use of these cells for targeted delivery might be preferable to multiple stereotactic injections for the delivery of molecules that require distribution throughout the brain, such as for enzyme replacement in lysosomal storage diseases[12]. Another characteristic of neural stem cells that makes them attractive vehicles for targeted delivery is their tropic behaviour toward neoplasms, which could be exploited to target minute brain metastases and infiltrating malignant satellites after main tumour resection.

## Neural stem cell pathotropism

Neural stem cells (endogenous and transplanted) seem to be attracted to various experimental brain lesions of disparate aetiologies, such as cancers and areas of neurodegeneration. For example, neural stem cells have shown tropism toward gliomas and toward degenerating spinal cord motor neurons in a transgenic mouse model of amyotrophic lateral sclerosis[11,13,14] (ALS, see TABLE 1). Neural stem cell cancer tropism is not limited to primary brain malignancies and has also been observed in the periphery[15].

The fate of neural stem cells in the presence of lesions is not well understood, because most preclinical studies use only surrogate markers (for example, reduction in tumour burden or survival time after treatment)[9,16]. In certain pathologies, such as stroke lesions, transplanted cells appear to form astrocytes and neurons[17]. A glial fate may not be ideal, but might be preferable to neuronal differentiation that could participate in abnormal, possibly damaging, circuits.

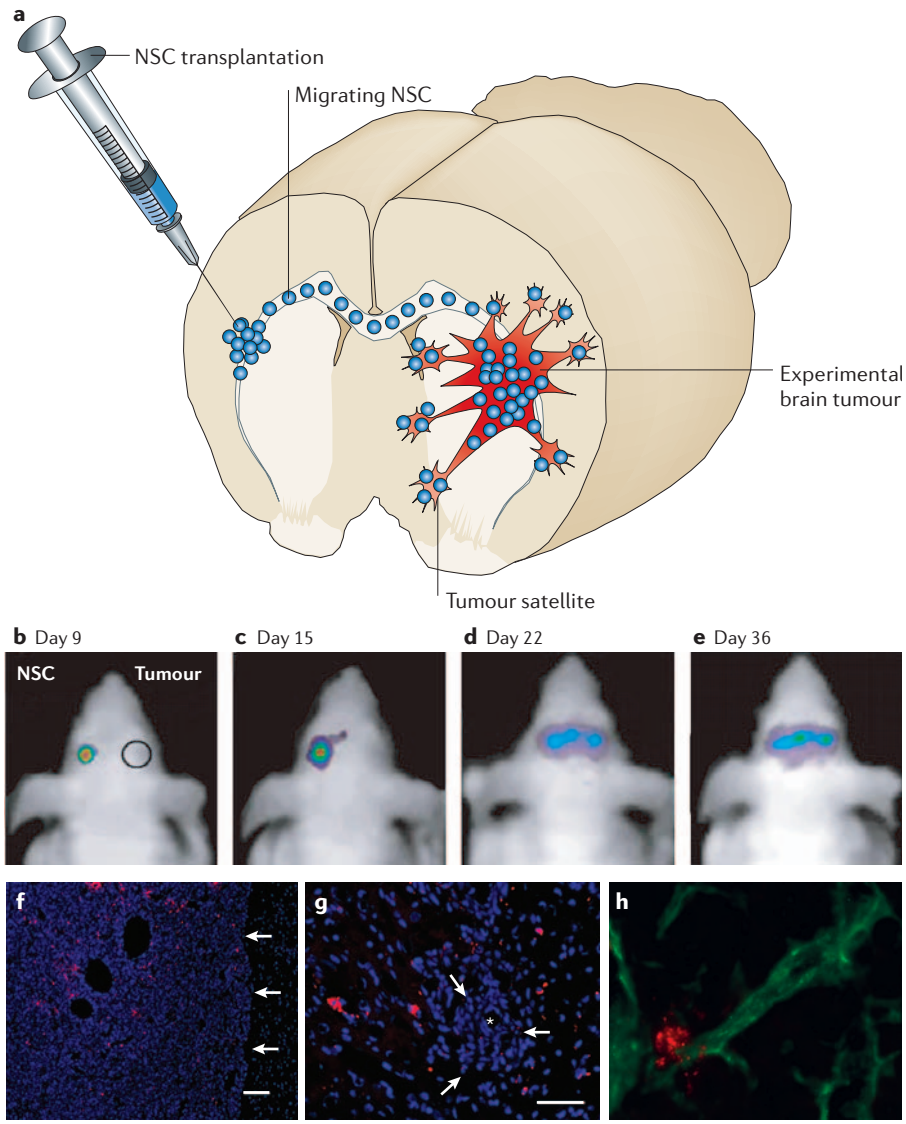


Figure 1 | **Neural stem cell homing in brain tumours. a** | Transplanted neural stem cells (NSCs) showing tropism for malignant cells in rodent models of brain neoplasia. **b–e** | Exogenous neural stem cells implanted at sites distant from experimental brain tumours have been shown to home to lesions. It is believed that this phenomenon could be exploited to track down widespread metastatic CNS pathology and to deliver antineoplastic therapeutic systems into brain malignancies. Panels show a time series for murine neural-stem-cell-like cells (C17.2 cell line) transfected with the 'luciferase' gene (*Luc*) and implanted into one hemisphere of experimental brain tumour-bearing mice. Photon emission imaging of *Luc* expression for these animals is shown on day 9, day 15, day 22 and day 36 (REF. 82). Migration towards the experimental tumour (black circle in **b**) was evident from day 15. **f–h** | Pathotropism of human neural-stem-cell-like cells (HB1.F3 cell line)[2]. **f** | Distribution of the cells (red) within a U87 (a glioblastoma cell line) xenograft (arrows indicate tumour border; cell nuclei shown in purple). **g** | Distant tumour satellite of a U251 (a glioblastoma cell line) xenograft, surrounded by HB1.F3 cells (a blood vessel is marked by an asterisk). Note that neural stem cells can migrate transcallosally from one hemisphere to the other and also infiltrate small tumour satellites that have dislodged from the main tumour mass. **h** | Proximity of a HB1.F3 cell (red) to a blood vessel (green) in a U87 xenograft. Panels **b–e** reprinted, with permission, from REF. 82 © (2003) Macmillan Magazines Ltd. Panels **f–h** reprinted, with permission, from REF. 23 © (2005) Neoplasia.

The normal course of neural stem cell development and migration *in vivo* is controlled primarily by the microenvironment in regions of the brain that harbour neural stem cells. The microenvironments surrounding neural stem cells are populated by astroglia, microglia and endothelial cells, which are important regulators of stem cell generation, migration and differentiation during maintenance of brain homeostasis[18–22]. Disturbances in the environment due to brain pathology can profoundly affect stem cell behaviour by disrupting the environmental equilibrium and exposing the cells to factors that they do not normally encounter. For example, gradients of factors such as vascular endothelial growth factor (VEGF) or stromal cell-derived factor 1 (SDF1), which emanate from distant brain lesions, may act as attractants for stem cells[23,24].

In attempting to predict the behaviour of stem cells in the brain, it is important to consider both endogenous stem cells and those that are transplanted to the brain. Endogenous and transplanted neural stem cells are often found to respond similarly to pathology, but there are some important differences to keep in mind. Cultured neural stem cells used for transplantation have been expanded in culture well beyond their expected proliferative capacity *in vivo*. Because culture conditions have a strong influence on the phenotype of cells, culture could markedly alter the cells' response to their environment when reintroduced *in vivo*. The results of a recent study showed that exposure to the mitogen epidermal growth factor (EGF) may confer an invasive and more 'stem-like' phenotype to neuronal progenitors[4]. Until more is known about the receptors expressed by neural stem cells *in situ* and their effects on genetic, epigenetic, transcriptional and translational levels, information about exogenous populations needs to be applied cautiously to interpretations of endogenous stem cell behaviour[5,6,8].

The molecular basis of neural cell pathotropism is not well understood and different pathologies may involve different factors. Cultured neural stem cells express a wide variety of receptors that should enable them to respond to many chemotactic signals that emanate from brain pathologies (TABLE 2). Experimental studies show that they home to localized and widespread lesions after transplantation (TABLE 1). Some factors that can be held responsible for this phenomenon belong to the chemokine superfamily[24–26] (chemotactic cytokines; TABLE 2). Chemokine and cytokine production is a common feature of many brain lesions, including stroke and brain malignancy, which suggests that these factors could be important in mediating the responses of stem cells to pathology[24–26].

## Regulation of neural stem cell tropism

The available information suggests that there are at least three important physiological processes that influence the migratory behaviour of transplanted neural stem cells: inflammation, reactive astrocytosis and angiogenesis (FIG. 3).

***Inflammation.*** *In vitro*, microglia can induce neural stem cell migration[22,27]. It is an attractive hypothesis that the inflammatory response to brain pathology is the common denominator responsible for the seemingly uniform attraction of stem cells to disparate brain lesions. The prevailing view, based on studies of multiple sclerosis, epilepsy, lipopolysaccharide-induced encephalitis and brain irradiation, is that brain inflammation is detrimental to the CNS in general and neurogenesis in particular[28–31]. Microglia are the first line in defence against brain pathologies, functioning as a damage surveillance system in the brain and responding to insults by producing cytokines, which, in turn, initiate further reactive responses[32]. However, activated immune cells also release neurotrophins[29,33], which would be expected to protect neurons, and microglial cells or inflammatory cytokines can modulate the mobilization of neural stem cells both *in vitro* and *in vivo*. This suggests that microglia might have a role in initiating and coordinating neural-stem-cell-based brain repair mechanisms[22,27,29,34].

***Reactive astrocytosis.*** As inflammatory cytokines are released by microglia in response to a pathological process, reactive astrocytosis, characterized by hyperplasia, hypertrophy and an increase in glial fibrillary acidic protein (GFAP), follows[35,36]. Triggers and mechanisms of this multifaceted response are not fully understood, but some factors known to be involved are the proximity of the astrocytes to a CNS pathology, the type of lesion and the types of cytokine produced (for example, interleukin-1β (IL-1β))[35,37].

Studies of the acute effects of inflammatory signals suggest that certain types of activated astrocyte promote neuronal survival, tissue regeneration and stem cell migration[38–40] (FIG. 4). For example, reactive astrocytes in the stroke penumbra secrete SDF1, which is at least partially responsible for the attraction of neural stem cells to these lesions[24]. Adult astrocytes are also involved in the guidance of leukocyte and glial homing toward brain injuries[41,42] and can revert in response to soluble factors invoked by tissue damage to a phenotype resembling radial glia in the developing brain[43]. These cells can support directed neural progenitor migration[43]. Although some types of glial activation might have beneficial effects, it is important to note that certain reactive astrocytes are thought to interfere with neuronal–glial signalling and impede neural progenitor homing by forming glial scars and secreting factors such as slit homologue 2 (SLIT2), tumour necrosis factor-α (TNFα) and high molecular weight hyaluronic acid[44–46].

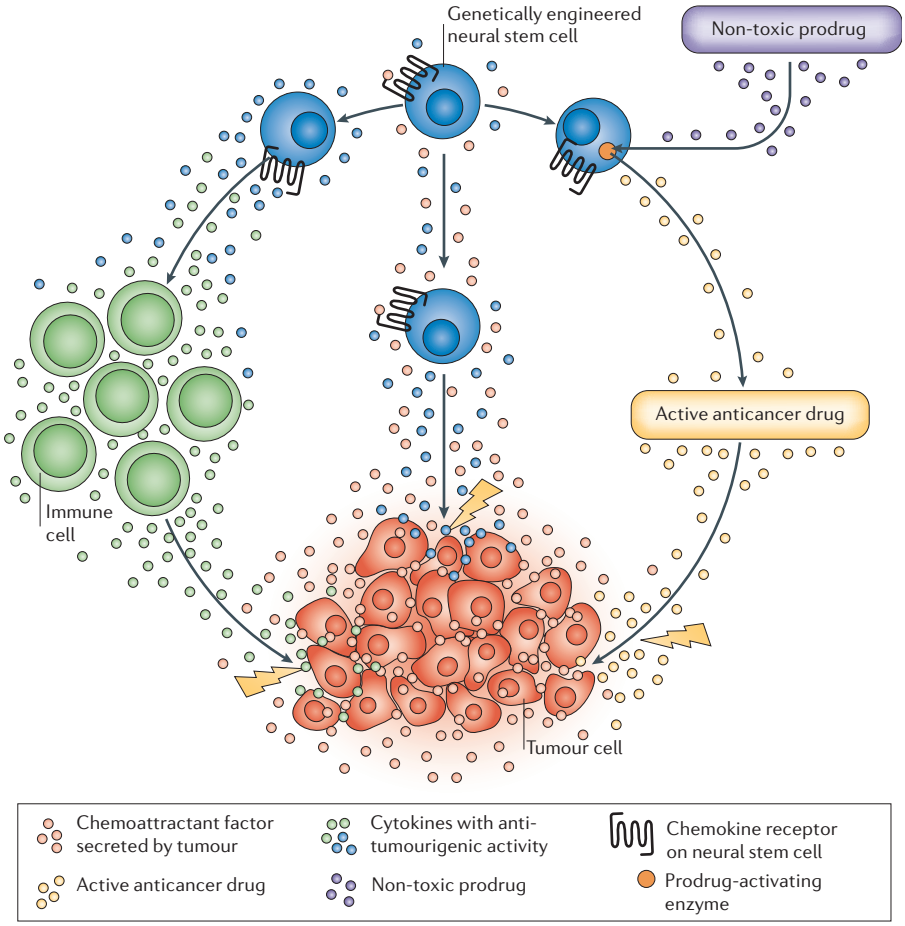


Figure 2 | **Determinants of neural stem cell homing to brain tumours for delivery of gene therapy.** Neural stem cells express various receptors for chemoattractant signals as a result of brain pathology. Among these chemoattractants are chemokines such as stromal cell-derived factor 1 (SDF1, also known as chemokine (C–X–C motif) ligand 12, CXCL12) and monocyte chemoattractant protein 1 (MCP1, also known as chemokine (C–C motif) ligand 2, CCL2) or other chemotactic proteins such as vascular endothelial growth factor (VEGF). Stem cells can be genetically engineered to express enzymes that metabolize non-toxic prodrugs locally, thereby allowing production of the active form of the drug or expression of cytokines that act directly on the tumour or activate immune cells, which, in turn, attack the tumour.

***Angiogenesis.*** Evidence is emerging for an intimate relationship between CNS morphogenesis and endothelial cells[47,48]. The basal lamina produced by endothelial cells contains many components that are believed to be important for the maintenance of a neurogenic niche[21,49]. Therefore, endothelial cells could also be involved in the regulation of the response of neural stem cells to pathology. Vasculogenesis resulting from brain pathology could enhance neural stem cell mobilization by producing chemoattractants such as VEGF. VEGF-mediated homing of stem cells might have a key role in brain development as well as neural stem cell glioma tropism[23,50,51]. In addition, SDF1 is expressed by endothelial cells as well as astrocytes in stroke lesions and could be important for attraction of neural stem cells[24]. Because neural stem cells seem to interact with pathology-activated endothelial cells from the luminal side, adhering and transmigrating in a similar fashion to leukocytes[52,53], it is possible that they can be delivered via the bloodstream. In support of this idea, a recent report shows that neural stem cells injected into the bloodstream in a mouse model of multiple sclerosis establish atypical niches around blood vessels, where they remain in an undifferentiated state and appear to suppress the inflammatory process[53,54].

Table 1 | **Disease models with neural stem cell tropism**

| Animal model | Human disorder | Refs |
|---|---|---|
| ***Neoplastic lesion*** | | |
| Glioma graft | Primary brain malignancy | 11,13 |
| Lung carcinoma vasculature | Malignancy | 83 |
| Breast tumour, prostate cancer, malignant melanoma | Brain metastases, malignancy | 15 |
| ***Chronic/degenerative lesion*** | | |
| Intrastriatal 6-OHDA lesion model | Parkinson's disease | 84,85 |
| MPTP-lesioned aged mice | Parkinson's disease | 55 |
| Selective cortical motor neuron ablation | Neurodegeneration | 86 |
| Mutant superoxide dismutase 1 transgenic mouse (TgN(SOD1-G93A)1Gur) | Amyotrophic lateral sclerosis | 14 |
| ***Metabolic lesion*** | | |
| β-Glucuronidase-deficient mouse | Sly disease (MPS VII) | 78 |
| Acid sphingomyelinase-knockout mouse | Type A Niemann–Pick disease | 87 |
| ***Acute/traumatic lesion*** | | |
| Middle cerebral artery occlusion | Stroke | 17 |
| Rice–Vanucci model | Neonatal stroke | 88 |
| Traumatic brain injury | Traumatic brain injury | 89,90 |
| ***Inflammatory lesion*** | | |
| Experimental allergic encephalomyelitis | Multiple sclerosis | 53,54,91,92 |
| MHV-MS | Multiple sclerosis | 93 |

6-OHDA, 6-hydroxydopamine; MHV-MS, mouse hepatitis virus model of multiple sclerosis; MPS, mucopolysaccharidosis; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

## Choosing a vehicle for delivery

There is considerable diversity among neural stem cell lines and they may not all be equally suited for therapy. The ideal neural stem cell delivery vehicle would be stable in tissue culture and capable of sustained, preferably regulated, expression of therapeutic molecules. The cells should have predictable and appropriate differentiation patterns in culture and after transplantation, and survive long term *in vivo* without forming tumours. For the therapeutic strategy to be effective, the neural stem cells chosen should demonstrate responsiveness to the chemotactic signals produced by the type of pathology that they are used to treat. Ideally, there would be a means for facile delivery of the cells (for example, via the bloodstream).

### *Should cell lines be immortalized?*

Historically, non-tumour cells had to be immortalized to expand specific cell types sufficiently to facilitate their characterization. Immortalizing cells usually involves introduction of an oncogene that allows the cells to expand beyond the time at which they would normally reach senescence. Identification of growth factors that enhance long-term culture of non-immortalized neural stem cells has made immortalization less necessary, and there are concerns about the utility of immortalized cells as research tools and in clinical settings. Immortalized neural stem cells appear to have characteristics that are atypical of most neural stem cell populations, such as extraordinary migratory abilities *in vivo* (for examples, compare REFS 55,56), and a higher degree of multipotency, which may increase the probability of tumour formation by fast growing stem cells[57,58].

However, there are some cases in which oncogene immortalization is an asset. Safety concerns may be mitigated by the therapeutic value of the more pronounced invasiveness and migratory capabilities of immortalized neural stem cells. Pragmatically, immortalization can allow propagation of cells with definable properties almost indefinitely, so that clonal populations with particular traits can be established. Furthermore, if immortalized cells could be shown to be reliably non-malignant, it would be much easier to control their quality than the quality of primary cell preparations for use in clinical settings, because the cell lines could be subjected to much more thorough analysis.

***Primary cells for transplantation.*** Although it can be argued that the ideal cell type for transplantation would be as similar to the endogenous neural stem cell as possible, there are some serious limitations to the use of primary cells. Unlike haematopoietic stem cell reconstitution of bone marrow, it is unlikely that a single neural stem cell or small group of cells would be sufficient to regenerate damaged brain tissue, so expansion of cells in culture will be required. How much expansion will be needed to produce a sufficient number of viable stem cells for a successful transplant has to be determined empirically for each disease.

*In vitro* culture creates its own problems. There are many neural stem cell lines and preparations in use, and each has been derived under different conditions from diverse sources and maintained under a wide variety of culture conditions. If neural stem cells are to be used clinically, an important challenge will be for investigators to agree on a common set of culture conditions and characterization criteria.

Table 2 | **Cytokines potentially involved in neural stem cell pathotropism**

| Receptor/ligand | Possible function in neural stem cells | Refs |
|---|---|---|
| CCR2/CCL2 (MCP1) and other chemokines | Migration | 26,94 |
| CCR3/various chemokines | Migration? | 95 |
| CCR5/various chemokines | Migration? | 94 |
| CXCR4/CXCL12 (SDF1) | Survival, migration, extravasation | 24,25,83 |
| CXCR1/CX3CL1 (fractalkine) | Survival, migration? | 94,95 |
| c-KIT/SCF | Proliferation, migration | 97,98 |
| VEGFR1 and R2/VEGF | Proliferation, migration | 23,99 |

CC/CXC/CXC3C, names for chemokines (ending -L) and chemokine receptors (ending -R) following the systematic nomenclature based on the position of the ligands' first two cysteine residues; c-KIT, kit oncogene; MCP1, monocyte chemoattractant protein 1; SCF, stem cell factor; SDF1, stromal cell-derived factor 1; VEGF, vascular endothelial growth factor; VEGFR1/2, VEGF receptor 1/2.

***Human embryonic stem cell-derived cells: can they be effective and safe?*** Human embryonic stem (ES) cells have several powerful advantages over other types of stem cell for therapeutic approaches. ES cells are cell lines derived from the 30–50 cells of the inner cell mass of blastocyst-stage embryos. Unlike neural stem cells, differentiation of ES cells is not limited to elements of the nervous system. ES cells are also immortal and do not undergo senescence after long periods of time in culture. Perhaps most importantly, the focused efforts to characterize ES cells in many laboratories means that all researchers can start with the same well-studied cell populations. This reduces the problems of reproducibility and quality control that have plagued studies with other stem cells. Human ES cells can be induced to differentiate along neuronal lineages[59], and isolated at a stage at which they resemble somatic neural stem cells. However, there are several important challenges that are unique to ES cell-based therapeutic approaches. First, we have to anticipate that because they have not experienced normal developmental cues, ES-derived cells might not develop conventional cellular phenotypes, and this may result in unpredictable outcomes *in vivo*[56]. At present, not much data are available on the migratory potential of human ES-cell-derived neural stem cell preparations compared with other neural stem cells. Furthermore, populations of ES-derived transplanted cells must be shown to be free of the tumourigenic cells that characterize undifferentiated populations. It is also important to keep in mind that the use of ES cells sometimes evokes ethical concerns associated with the derivation of these cells from early embryos.

***How will we decide?*** The decision for or against a certain cell preparation must be based on the medical principle of doing no harm, and the modern concept of evidence-based medicine. At present, a widely held view is that primary and ES-derived cells will be the most relevant therapeutic systems for targeting brain pathologies. However, there is insufficient evidence to clearly favour any cell type, and there is a need for comprehensive studies that compare different cell populations in the same preclinical setting[3]. New approaches, such as improved *in vivo* cell tracking tools, will be important for resolution of this issue[60,61].

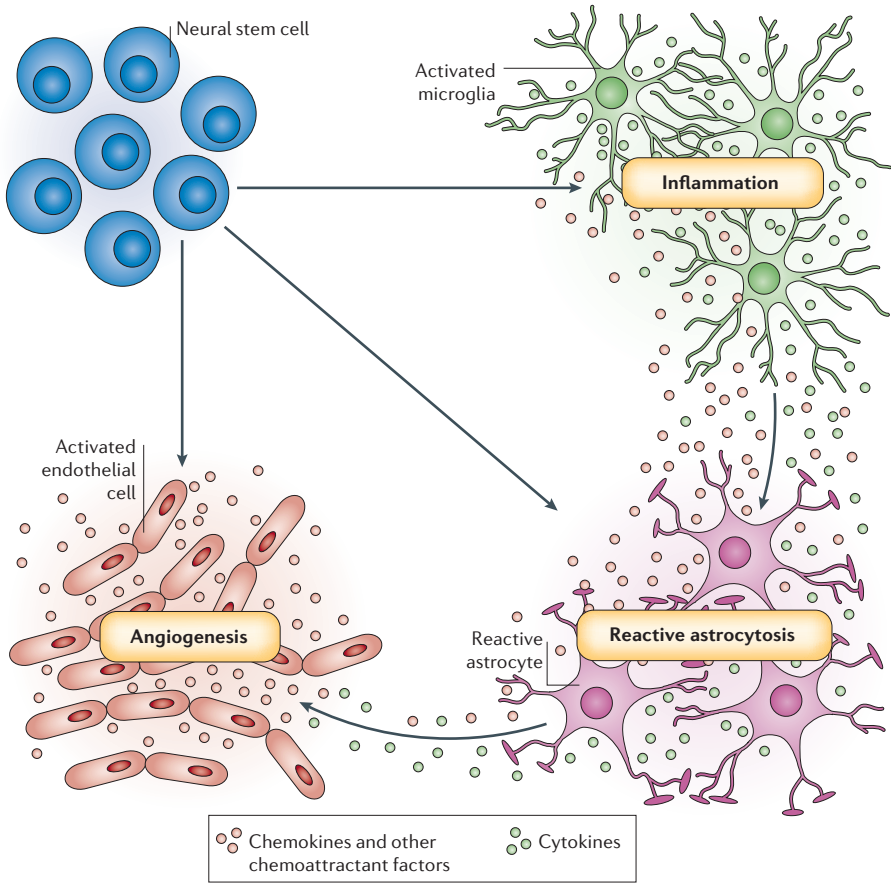


Figure 3 | **Determinants of neural stem cell pathotropism.** Neural stem cells are attracted by at least three physiological processes that are common to many brain pathologies: inflammation, reactive astrocytosis and angiogenesis. Pathology-induced CNS inflammation is mediated by activated microglia that release cytokines and chemokines, which, in turn, increase the inflammatory reaction (for instance, the cytokines interleukin-1β, IL-1β, interleukin-6, IL-6, and monocyte chemoattractant protein 1, MCP1, also known as chemokine (C–C motif) ligand 2, CCL2). MCP1 gradients can also attract neural stem cells. The brain lesion and subsequent inflammation trigger reactive astrocytosis. In response to signals emanating from inflammation, activated astrocytes secrete chemotactic factors (for example, stromal cell-derived factor 1, SDF1, also known as chemokine (C–X–C motif) ligand 12, CXCL12, and vascular endothelial growth factor, VEGF). VEGF and SDF1 can act both as chemoattractants for neural stem cells and as promoters of pathology-induced angiogenesis. The lesion-induced angiogenesis and inflammation-activated endothelial cells enhance neural stem cell homing to brain pathology by secreting chemoattractant factors (such as SDF1), and also offer an atypical, perivascular niche for support of immigrating neural stem cells.

## Neural stem cell-based gene therapy

In the nervous system, replacement of neurons is often considered to be the main goal of cell therapy. But cells, including stem cells, are already being used as gene delivery tools and for rescuing neurons rather than replacing them. The general rationale for gene therapy is that diseases that are caused by the lack of some crucial protein can be treated by restoring the protein using appropriate gene expression vectors. This idea was originally proposed for hereditary diseases such as lysosomal storage diseases[62]. In these diseases, a mutated catabolic enzyme causes a metabolic logjam in the upstream pathways and consequently floods the affected cells and surrounding tissue with accumulating substrates and toxic side products. In theory, the targeted therapeutic expression of a functional replacement gene in or near the affected area would restore the metabolic pathway. This originally well-defined concept has been broadened to include any genetic manipulation of cells or tissue to treat a disease. For example, proposed gene therapies for Alzheimer's disease include targeted expression of choline acetyltransferase to compensate for deficits in acetylcholine, which therefore results in the localized delivery of small molecules, in this case acetylcholine[63]. Exactly how stem cells will be used therapeutically depends on the nature of the disease or damage that requires treatment.

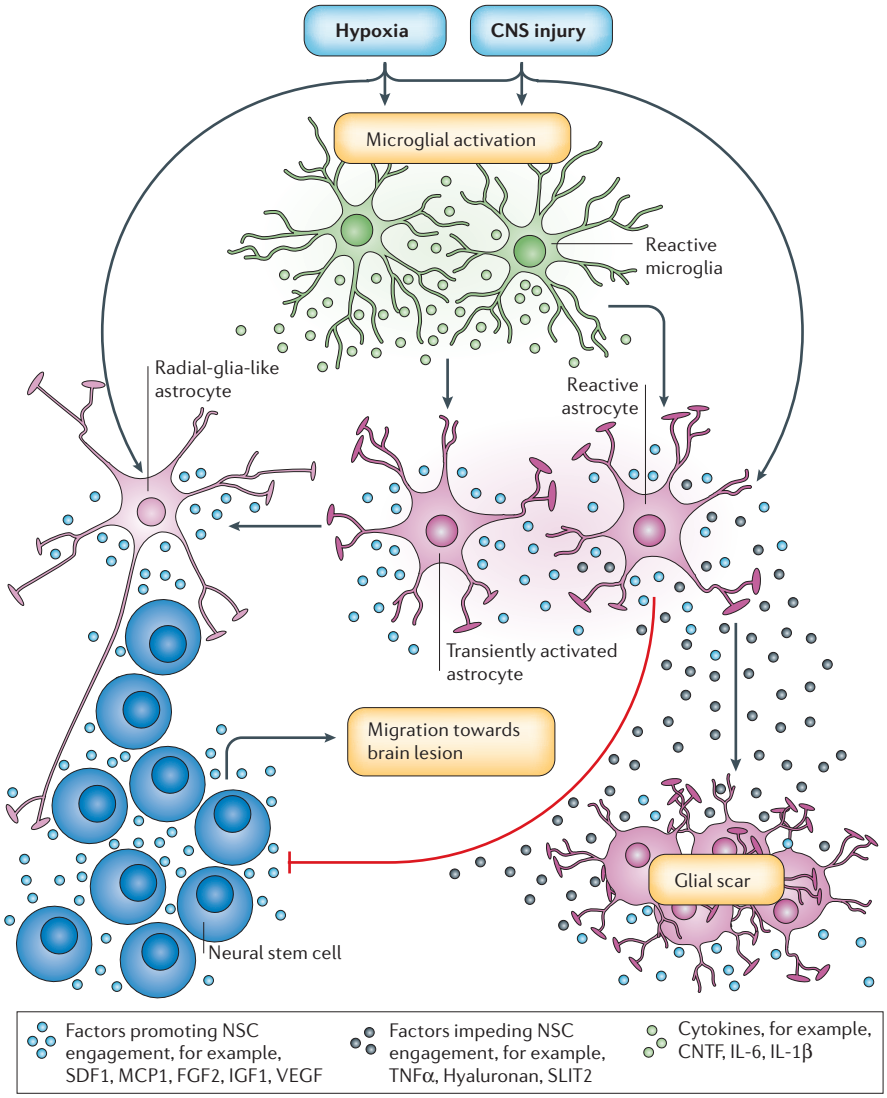


Figure 4 | **Model of activated astrocyte mediation of neural stem cell homing to brain pathology.** CNS injury, hypoxia, microglial activation and the subsequent release of inflammatory cytokines (such as interleukin-1β (IL-1β), IL-6 and ciliary neurotrophic factor (CNTF)) invoke complex responses known collectively as gliosis[3]. As a consequence of brain injury, some mature glia revert to a developmental, radial-glia-like state, and can directly mediate neural progenitor migration towards brain lesions. Cytokine release also causes transient activation of astrocytes. These transiently activated astrocytes are a source of chemoattractants (such as stromal cell-derived factor 1 (SDF1), vascular endothelial growth factor (VEGF) and monocyte chemoattractant protein 1 (MCP1)) that act on neural stem cells (NSCs)[3–6]. SDF1 may direct neural stem cell chain migration toward brain pathology along non-stereotypical routes[4]. Other factors (such as fibroblast growth factor 2 (FGF2) and insulin-like growth factor 1 (IGF1)) supplied by reactive astrocytes support neural stem cell proliferation, survival and differentiation[3,7]. Other astrocytes proliferate reactively, become hypertrophic and increase their glial fibrillary acidic protein (GFAP) content. This eventually results in the formation of a tightly compacted astrogliotic scar, which is the source of factors (such as slit homologue 2 (SLIT2), tumour necrosis factor-α (TNFα) and hyaluronan) that repel neural stem cells and might limit progenitor cells' regenerative capacity[3,8,9].

Neural stem cells can be genetically transduced *in vitro* and *in vivo*[12,64]. Currently, the most efficient and popular way of introducing genes into neural stem cells is by means of lentiviral vectors; the chief concerns about this approach are frequent transgene silencing *in situ* and that integration of the transgene can activate a nearby oncogene, leading to selection of abnormally growing subclones[58,65,66].

To highlight various stem cell-mediated gene delivery strategies, we discuss in more detail six examples of disorders of the nervous system that may benefit from such therapy.

***Parkinson's disease.*** A lack of dopamine in the putamen (caused by the degeneration of innervating neurons in the substantia nigra) has a central role in the pathogenesis of Parkinson's disease (PD). Systemic L-DOPA ($3,4(OH)_2$-phenylalanine) therapy is an effective treatment for the symptoms of PD early in the course of the disease, but does not prevent the continuation of degeneration and eventually becomes ineffective. Because the degeneration is relatively localized, PD was one of the first targets for cell therapy, and experimental transplants of fetal dopamine neurons into the putamen were pioneered in the 1980s. In these patients, the successful transplants seemed to work as dopamine pumps, similar to the systemic administration of L-DOPA, with the chief advantage of a transplant being a smoothing of the on–off cycle of symptoms, in which patients cycle through periods of being unable to move and periods of uncontrollable movement[67,68]. A concern about this approach is the patient-to-patient variability, which is partly due to the inconsistency of the fetal tissue used for transplant and is also dependent on intrinsic characteristics of the disease in each patient; in a controlled study, the best therapeutic benefit of transplants was on a par with the best achievable symptomatic improvement using L-DOPA in the same patient[68]. The mechanisms by which the transplants act are not clear; because of the paucity of functional connections in many transplants, it has been proposed that the transplanted cells were acting more as gene therapy vehicles for dopamine delivery than as replacement neurons. But the effects of fetal transplants might not be limited to acting as dopamine pumps; in some cases, functional connections have been observed, and it has been suggested that the transplanted cells may produce trophic factors that help to protect remaining dopaminergic neurons. Active preclinical investigations are testing the use of genetically induced production of neurotrophic factors such as glial cell line-derived neurotrophic factor (GDNF) or VEGF in neural stem cell transplants[69–71] (see also the following two paragraphs on neurotrophic factor delivery in neurodegenerative diseases).

***Alzheimer's disease.*** Alzheimer's disease presents a greater therapeutic challenge than PD, because the degeneration of neurons is widespread, beginning in the hippocampus, cortex and amygdala, and progressing to other regions of the brain. Current strategies for cell and gene therapy are focused on using cells to deliver neurotrophic factors.

Table 3 | **Examples of studies on neural stem cell-based gene therapy in animal models**

| Stem cells (source) | Route of administration | Disease model | Therapeutic strategy | Outcome | Refs |
|---|---|---|---|---|---|
| ***Alzheimer's disease*** | | | | | |
| HiB5–E8 | Intracerebral transplantation into medial septum and NBM | Transplantation: middle-aged rats<br>Memory tests: aged rats | Overexpressed NGF | Improved memory tasks at old age, less cholinergic atrophy | 96 |
| HiB5–human BDNF (rat) | Intracerebral transplantation into striatum and NBM | Intact rat brain | Overexpressed BDNF | Cholinergic hypertrophy | 100 |
| Embryonic cells from septal fetal nuclei | Intrahippocampal transplantation | Fornix lesion in rats | Endogenous ChAT activity | Behavioural recovery in memory tasks | 101 |
| ***Brain malignancy*** | | | | | |
| C17.2 (mouse) | Intratumoural transplantation | Rat glioma line (CNS1) in nude mice | HSV-1tk vector | Not tested | 10 |
| C57.npr.IL-4 (mouse) | Intratumoural transplantation | Mouse glioma cell line (GL261) in C57/B6 mice | IL-4 | Extended survival | 9 |
| ST14A.IL-4.3 (rat) | Intratumoural transplantation | Rat glioma cell line (C6) in Sprague–Dawley rats | IL-4 | Extended survival | 9 |
| C17.2-CD (mouse) | Intracranial transplantation: C17.2 mixed with glioma cells | Rat glioma cell lines (CNS-1, D74) and in rats and nude mice | CD (5-FC i.p.) | ~80% reduction in tumour volume | 11 |
| NSC-IL-12 (mouse) | Intratumoural transplantation | Mouse glioma cell line (GL26) in C57/B6 mice | IL-12 | Extended survival | 102 |
| NSC-TRAIL (mouse) | Intratumoural transplantation | Human glioma cell line (U343MG) in nude mice | TRAIL | Tumour size reduction | 76 |
| ST14A (rat) | Intracranial transplantation: ST14A mixed with C6 cells | Rat glioma cell line (C6) in Sprague–Dawley rat | CD (5-FC i.p.) | ~50% reduction in tumour growth | 103 |
| Human NSC and MDNCC (human) | Intracranial transplantation: distant to tumour | Human glioma cell line (U87-MG) in nude mice | sPF4 TRAIL | Reduced vascularization<br>Extended survival | 104 |
| NSCtk (rat) | Intratumoural transplantation: NSCtk cells mixed with C6 cells and after establishment of tumour | Rat glioma cell line (C6) in nude mice and in rats | HSVtk (GCV i. p.) | 100% survival when co-implanted, 70% after establishment of tumour | 16 |
| C17.2-FL-sTRAIL (mouse) | Intracerebral transplantation | Human glioma cell line (Gli26) in nude mice | TRAIL and luciferase | Decrease in tumour burden | 105 |
| H1B-F3-PEX (human) | Intracerebral transplantation | Human glioma cell line (U87-MG) in nude mice | PEX | 90% decrease in tumour burden | 77 |
| ***Injury-induced neuropathic pain*** | | | | | |
| RN46A-B14/V1, RN33B and 33G10.17 (rat) | Intrathecal transplantation | CCL-model in rats | 5-HT secretion, BDNF overexpression and GAD67 overexpression | Improvement of pain-related behaviour<br>GAD/GABA system modulation | 106<br>107<br>108 |
| 33GAL.19 (rat) | Intrathecal transplantation | CCL-model in rats | Overexpressed galanin | Improvement of pain-related behaviour | 109 |
| 33BDNF.4 (rat) | Subarachnoidal injection | CCL-model in rats | Overexpressed BDNF | Improvement of allodynia | 110 |
| Spinal cord NPC (rat) | Intrathecal injection | CCL-model in rats | Endogenous GAD-competence | Improvement of allodynia | 111 |
| RN46A-B14/V1 (rat) | Subarachnoidal injection | Hemisection model of chronic central pain in rats | 5-HT and overexpressed BDNF | Improvement of locomotor function and allodynia | 80 |
| Spinal cord NSC (rat) | Intraspinal injection | Spinal cord contusion | Native NSC/NSC directed into oligodendrocyte fate with NGN2 overexpression | Improvement of locomotor function and allodynia | 81 |

5-FC, 5-fluorocytosine; 5-FU, 5-fluorouracil; 5-HT, 5-hydroxytryptamine (serotonin); BDNF, brain-derived neurotrophic factor; CCL-model, chronic constriction injury of the sciatic nerve model of chronic pain; CD, cytosine deaminase; ChAT, choline acetyl transferase; CNS1, conserved non-coding sequence 1; GABA, γ-aminobutyric acid; GAD, glutamic acid decarboxylase; GCV, gancyclovir; HiB5, rat hippocampus transformed SV40 large T antigen cell line; HSV-1, herpes simplex virus 1; IL-4 , interleukin-4; i.p., intraperitoneal; MDNCC, marrow-derived neural-competent cells; NBM, nucleus basalis Meynert; NGF, nerve growth factor; NGN2, neurogenein 2; NPC, neural progenitor cells; NSC, neural stem cells; NSPC, neural stem/progenitor cells; PEX, carboxy-terminal homeopexin-like domain containing fragment of matrix metalloproteinase 2; sPF4, secreted platelet factor 4; tk, thymidine kinase; TRAIL, tumour necrosis factor-related apoptosis-inducing ligand.

Neurotrophic factors are thought to protect neurons from degeneration and to re-activate impaired circuitry in neurodegenerative diseases; the first phase of a clinical trial using fibroblasts to deliver nerve growth factor (NGF) has recently been completed[72]. An extension of this trial is using adeno-associated virus (AAV) to deliver NGF expression vectors directly to the brain.

Most recently, we have proposed that the homing qualities of neural stem cells might be exploited to directly target amyloid plaques with therapeutic enzymes (F.-J.M. and J.F.L., unpublished observations).

***Amyotrophic lateral sclerosis.*** Experimental studies show that overexpression of growth factors such as GDNF, insulin-like growth factor 1 (IGF1) or VEGF can have beneficial effects on the course of ALS in animal models[73,74]. The challenge for adapting this sort of therapy for clinical use is the delivery of these large molecules across the blood–brain barrier. So far, viral vectors seem to be the best means of increasing the production of these factors *in situ*. Cell therapy might be a more effective means of delivering these large proteins to specific areas of the CNS where they can aid in the survival of neurons[75].

***Brain malignancy.*** Neural stem cells seem to be attracted to certain brain tumours and this characteristic can be exploited, allowing these cells to be used for local chemotherapy (FIG. 2). The main issues under investigation in preclinical studies are the optimal choice of stem cell type, and the most effective therapeutic system to use (TABLE 3). So far, most studies have used immortalized neural stem cell-like cells in preclinical models. The large variety of therapeutic systems tested encompasses lytic viruses, prodrug-converting enzymes, immunomodulatory cytokines, proteins with anti-angiogenic activity and cytokines with direct anti-tumoural activity[9–11,76,77].

***Disorders of brain metabolism.*** Inborn genetic defects affecting the CNS, such as lysosomal storage diseases, are some of the most promising targets for stem cell therapy. Dispersion of genetically normal stem cells in the brain would allow widespread delivery of missing enzymatic activities[12,78]. The current preclinical research objectives for this type of therapy are the optimal timing of treatment, which could be *in utero*, and the type of stem cell to use. The most straightforward targets of this therapy are storage diseases that are frequently accompanied by an extremely prominent neuroinflammatory component such as that seen in galactocerebrosidase deficiency (Krabbe disease in humans and the twitcher mouse). Significant progress has been made recently in protecting neural stem cells from inflammatory damage and could be applied to this particular situation[30,31,79].

***Neuropathic pain.*** The delivery of cells and genes to treat certain forms of neuropathic pain is an active area of research (TABLE 3). Potentially therapeutic molecules such as growth factors and neurotransmitters delivered by stem cells or other cells seem to alleviate forms of chronic pain in animal models. An emerging conceptual aspect of these studies is that neural stem cells (and their derivatives — astrocytes and oligodendrocytes) might have another unexpected application; they may be useful for modulating injury-induced central pain by influencing neuronal circuitry and excitability[80,81].

From the pioneering work in PD to the emerging exploration of stem cell therapy for Alzheimer's disease, there is growing enthusiasm for the potential of stem cells for the treatment of various diseases of the nervous system. Neural stem cell-based drug delivery has the potential to maximize the therapeutic impact of drugs. However, most stem cell-based gene therapy concepts are still in the preclinical testing phase and will have to pass significant hurdles to become viable therapeutic options.

## Summary

Neural stem cells could be exploited as delivery vehicles for therapeutic molecules to treat CNS diseases. They show tropism towards brain pathology, which appears to be mediated at least in part by chemokines. The challenges for the clinical development of this approach are in determining which neural stem cells are appropriate for each application, what genes or chemicals can be delivered, and what diseases are suitable targets for this approach.

It is important to remember that the current dominant concept in this field predicts that neural stem cells are appropriate mainly for cell replacement therapy. Although experiments continue to be designed with the expectation of this result, the outcomes could yield surprising new interpretations. We will benefit from remaining receptive to unconventional concepts and unexpected results that will lead us to future discoveries that we cannot imagine today.

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1. Gage, F. H. Mammalian neural stem cells. *Science* **287**, 1433–1438 (2000).
2. Pevny, L. & Rao, M. S. The stem-cell menagerie. *Trends Neurosci.* **26**, 351–359 (2003).
3. Soares, S. & Sotelo, C. Adult neural stem cells from the mouse subventricular zone are limited in migratory ability compared to progenitor cells of similar origin. *Neuroscience* **128**, 807–817 (2004).
4. Doetsch, F., Petreanu, L., Caille, I., Garcia-Verdugo, J. M. & Alvarez-Buylla, A. EGF converts transit-amplifying neurogenic precursors in the adult brain into multipotent stem cells. *Neuron* **36**, 1021–1034 (2002).
5. Maslov, A. Y., Barone, T. A., Plunkett, R. J. & Pruitt, S. C. Neural stem cell detection, characterization, and age-related changes in the subventricular zone of mice. *J. Neurosci.* **24**, 1726–1733 (2004).
6. Mi, R. *et al.* Immortalized neural stem cells differ from nonimmortalized cortical neurospheres and cerebellar granule cell progenitors. *Exp. Neurol.* **194**, 301–319 (2005).
7. Lie, D. C., Song, H., Colamarino, S. A., Ming, G. L. & Gage, F. H. Neurogenesis in the adult brain: new strategies for central nervous system diseases. *Annu. Rev. Pharmacol. Toxicol.* **44**, 399–421 (2004).
8. Emsley, J. G., Mitchell, B. D., Kempermann, G. & Macklis, J. D. Adult neurogenesis and repair of the adult CNS with neural progenitors, precursors, and stem cells. *Prog. Neurobiol.* **75**, 321–341 (2005).
9. Benedetti, S. *et al.* Gene therapy of experimental brain tumors using neural progenitor cells. *Nature Med.* **6**, 447–450 (2000).
10. Herrlinger, U. *et al.* Neural precursor cells for delivery of replication-conditional HSV-1 vectors to intracerebral gliomas. *Mol. Ther.* **1**, 347–357 (2000).
11. Aboody, K. S. *et al.* Neural stem cells display extensive tropism for pathology in adult brain: evidence from intracranial gliomas. *Proc. Natl Acad. Sci. USA* **97**, 12846–12851 (2000).
12. Consiglio, A. *et al.* Robust *in vivo* gene transfer into adult mammalian neural stem cells by lentiviral vectors. *Proc. Natl Acad. Sci. USA* **101**, 14835–14840 (2004).
13. Glass, R. *et al.* Glioblastoma-induced attraction of endogenous neural precursor cells is associated with improved survival. *J. Neurosci.* **25**, 2637–2646 (2005).
14. Chi, L. *et al.* Motor neuron degeneration promotes neural progenitor cell proliferation, migration and neurogenesis in the spinal cords of ALS mice. *Stem Cells Express* 11 Aug 2005 (doi:10.1634/stemcells.2005-0076).
15. Brown, A. B. *et al.* Intravascular delivery of neural stem cell lines to target intracranial and extracranial tumors of neural and non-neural origin. *Hum. Gene Ther.* **14**, 1777–1785 (2003).
16. Li, S. *et al.* Bystander effect-mediated gene therapy of gliomas using genetically engineered neural stem cells. *Cancer Gene Ther.* **12**, 600–607 (2005).
17. Kelly, S. *et al.* Transplanted human fetal neural stem cells survive, migrate, and differentiate in ischemic rat cerebral cortex. *Proc. Natl Acad. Sci. USA* **101**, 11839–11844 (2004).
18. Song, H., Stevens, C. F. & Gage, F. H. Astroglia induce neurogenesis from adult neural stem cells. *Nature* **417**, 39–44 (2002).
19. Palmer, T. D., Willhoite, A. R. & Gage, F. H. Vascular niche for adult hippocampal neurogenesis. *J. Comp. Neurol.* **425**, 479–494 (2000).
20. Shen, Q. *et al.* Endothelial cells stimulate self-renewal and expand neurogenesis of neural stem cells. *Science* **304**, 1338–1340 (2004).
21. Mercier, F., Kitasako, J. T. & Hatton, G. I. Anatomy of the brain neurogenic zones revisited: fractones and the fibroblast/macrophage network. *J. Comp. Neurol.* **451**, 170–188 (2002).
22. Aarum, J., Sandberg, K., Haeberlein, S. L. & Persson, M. A. Migration and differentiation of neural precursor cells can be directed by microglia. *Proc. Natl Acad. Sci. USA* **100**, 15983–15988 (2003).

23. Schmidt, N. O. *et al.* Brain tumor tropism of transplanted human neural stem cells is induced by vascular endothelial growth factor. *Neoplasia* **7**, 623–629 (2005).
24. Imitola, J. *et al.* Directed migration of neural stem cells to sites of CNS injury by the stromal cell-derived factor 1α/CXC chemokine receptor 4 pathway. *Proc. Natl Acad. Sci. USA* **101**, 18117–18122 (2004).
25. Ehtesham, M. *et al.* Glioma tropic neural stem cells consist of astrocytic precursors and their migratory capacity is mediated by CXCR4. *Neoplasia* **6**, 287–293 (2004).
26. Widera, D. *et al.* MCP-1 induces migration of adult neural stem cells. *Eur. J. Cell Biol.* **83**, 381–387 (2004).
27. Ben-Hur, T. *et al.* Effects of proinflammatory cytokines on the growth, fate, and motility of multipotential neural precursor cells. *Mol. Cell. Neurosci.* **24**, 623–631 (2003).
28. Block, M. L. & Hong, J. S. Microglia and inflammation-mediated neurodegeneration: multiple triggers with a common mechanism. *Prog. Neurobiol.* **76**, 77–98 (2005).
29. Aharoni, R., Arnon, R. & Eilam, R. Neurogenesis and neuroprotection induced by peripheral immunomodulatory treatment of experimental autoimmune encephalomyelitis. *J. Neurosci.* **25**, 8217–8228 (2005).
30. Ekdahl, C. T., Claasen, J. H., Bonde, S., Kokaia, Z. & Lindvall, O. Inflammation is detrimental for neurogenesis in adult brain. *Proc. Natl Acad. Sci. USA* **100**, 13632–13637 (2003).
31. Monje, M. L., Toda, H. & Palmer, T. D. Inflammatory blockade restores adult hippocampal neurogenesis. *Science* **302**, 1760–1765 (2003).
32. Nimmerjahn, A., Kirchhoff, F. & Helmchen, F. Resting microglial cells are highly dynamic surveillants of brain parenchyma *in vivo*. *Science* **308**, 1314–1318 (2005).
33. Barouch, R. & Schwartz, M. Autoreactive T cells induce neurotrophin production by immune and neural cells in injured rat optic nerve: implications for protective autoimmunity. *FASEB J.* **16**, 1304–1306 (2002).
34. Wong, G., Goldshmit, Y. & Turnley, A. M. Interferon-γ but not TNF α promotes neuronal differentiation and neurite outgrowth of murine adult neural stem cells. *Exp. Neurol.* **187**, 171–177 (2004).
35. Fernaud-Espinosa, I., Nieto-Sampedro, M. & Bovolenta, P. Differential activation of microglia and astrocytes in aniso- and isomorphic gliotic tissue. *Glia* **8**, 277–291 (1993).
36. Wang, K. & Walz, W. Unusual topographical pattern of proximal astrogliosis around a cortical devascularizing lesion. *J. Neurosci. Res.* **73**, 497–506 (2003).
37. da Cunha, A. *et al.* Control of astrocytosis by interleukin-1 and transforming growth factor-β 1 in human brain. *Brain Res.* **631**, 39–45 (1993).
38. Spranger, M. *et al.* Regulation of nerve growth factor (NGF) synthesis in the rat central nervous system: comparison between the effects of interleukin-1 and various growth factors in astrocyte cultures and *in vivo*. *Eur. J. Neurosci.* **2**, 69–76 (1990).
39. Friedman, W. J. *et al.* Regulation of β-nerve growth factor expression by inflammatory mediators in hippocampal cultures. *J. Neurosci. Res.* **27**, 374–382 (1990).
40. Liberto, C. M., Albrecht, P. J., Herx, L. M., Yong, V. W. & Levison, S. W. Pro-regenerative properties of cytokine-activated astrocytes. *J. Neurochem.* **89**, 1092–1100 (2004).
41. Babcock, A. A., Kuziel, W. A., Rivest, S. & Owens, T. Chemokine expression by glial cells directs leukocytes to sites of axonal injury in the CNS. *J. Neurosci.* **23**, 7922–7930 (2003).
42. Wang, K., Bekar, L. K., Furber, K. & Walz, W. Vimentin-expressing proximal reactive astrocytes correlate with migration rather than proliferation following focal brain injury. *Brain Res.* **1024**, 193–202 (2004).
43. Leavitt, B. R., Hernit-Grant, C. S. & Macklis, J. D. Mature astrocytes transform into transitional radial glia within adult mouse neocortex that supports directed migration of transplanted immature neurons. *Exp. Neurol.* **157**, 43–57 (1999).
44. Hagino, S. *et al.* Slit and glypican-1 mRNAs are coexpressed in the reactive astrocytes of the injured adult brain. *Glia* **42**, 130–138 (2003).
45. Fawcett, J. W. & Asher, R. A. The glial scar and central nervous system repair. *Brain Res. Bull.* **49**, 377–391 (1999).
46. Back, S. A. *et al.* Hyaluronan accumulates in demyelinated lesions and inhibits oligodendrocyte progenitor maturation. *Nature Med.* **11**, 966–972 (2005).
47. Cleaver, O. & Melton, D. A. Endothelial signaling during development. *Nature Med.* **9**, 661–668 (2003).
48. Carmeliet, P. & Tessier-Lavigne, M. Common mechanisms of nerve and blood vessel wiring. *Nature* **436**, 193–200 (2005).
49. Alvarez-Buylla, A. & Lim, D. A. For the long run: maintaining germinal niches in the adult brain. *Neuron* **41**, 683–686 (2004).
50. Bagnard, D. *et al.* Semaphorin 3A–vascular endothelial growth factor-165 balance mediates migration and apoptosis of neural progenitor cells by the recruitment of shared receptor. *J. Neurosci.* **21**, 3332–3341 (2001).
51. Zhang, H., Vutskits, L., Pepper, M. S. & Kiss, J. Z. VEGF is a chemoattractant for FGF-2-stimulated neural progenitors. *J. Cell Biol.* **163**, 1375–1384 (2003).
52. Allport, J. R., Shinde Patil, V. R. & Weissleder, R. Murine neuronal progenitor cells are preferentially recruited to tumor vasculature via α4-integrin and SDF-1α-dependent mechanisms. *Cancer Biol. Ther.* **3**, 838–844 (2004).
53. Pluchino, S. *et al.* Injection of adult neurospheres induces recovery in a chronic model of multiple sclerosis. *Nature* **422**, 688–694 (2003).
54. Pluchino, S. *et al.* Neurosphere-derived multipotent precursors promote neuroprotection by an immunomodulatory mechanism. *Nature* **436**, 266–271 (2005).
55. Ourednik, J., Ourednik, V., Lynch, W. P., Schachner, M. & Snyder, E. Y. Neural stem cells display an inherent mechanism for rescuing dysfunctional neurons. *Nature Biotechnol.* **20**, 1103–1110 (2002).
56. Ruschenschmidt, C., Koch, P. G., Brustle, O. & Beck, H. Functional properties of ES cell-derived neurons engrafted into the hippocampus of adult normal and chronically epileptic rats. *Epilepsia* **46** (Suppl. 5), 174–183 (2005).
57. Gao, W. Q. & Hatten, M. E. Immortalizing oncogenes subvert the establishment of granule cell identity in developing cerebellum. *Development* **120**, 1059–1070 (1994).
58. Imren, S. *et al.* High-level β-globin expression and preferred intragenic integration after lentiviral transduction of human cord blood stem cells. *J. Clin. Invest.* **114**, 953–962 (2004).
59. Carpenter, M. K. *et al.* Enrichment of neurons and neural precursors from human embryonic stem cells. *Exp. Neurol.* **172**, 383–397 (2001).
60. Tang, Y. *et al. In vivo* tracking of neural progenitor cell migration to glioblastomas. *Hum. Gene Ther.* **14**, 1247–1254 (2003).
61. Hoehn, M. *et al.* Monitoring of implanted stem cell migration *in vivo*: a highly resolved *in vivo* magnetic resonance imaging investigation of experimental stroke in rat. *Proc. Natl Acad. Sci. USA* **99**, 16267–16272 (2002).
62. Gene Therapy *Wikipedia* [online], <http://en.wikipedia.org/wiki/Gene_therapy> (2005).
63. Pizzo, D. P., Coufal, N. G., Lortie, M. J., Gage, F. H. & Thal, L. J. Regulatable acetylcholine-producing fibroblasts enhance cognitive performance. *Mol. Ther.* 26 Sep 2005 (doi:10.1016/j.mythe.2005.08.001).
64. Bharali, D. J. *et al.* Organically modified silica nanoparticles: a nonviral vector for *in vivo* gene delivery and expression in the brain. *Proc. Natl Acad. Sci. USA* **102**, 11539–11544 (2005).
65. Kustikova, O. *et al.* Clonal dominance of hematopoietic stem cells triggered by retroviral gene marking. *Science* **308**, 1171–1174 (2005).
66. Vroemen, M., Weidner, N. & Blesch, A. Loss of gene expression in lentivirus- and retrovirus-transduced neural progenitor cells is correlated to migration and differentiation in the adult spinal cord. *Exp. Neurol.* **195**, 127–139 (2005).
67. Zurn, A. D., Tseng, J. & Aebischer, P. Treatment of Parkinson's disease. Symptomatic cell therapies: cells as biological minipumps. *Eur. Neurol.* **36**, 405–408 (1996).
68. Freed, C. R., Breeze, R. E., Fahn, S. & Eidelberg, D. Preoperative response to levodopa is the best predictor of transplant outcome. *Ann. Neurol.* **55**, 896; author reply 896–897 (2004).
69. Akerud, P., Canals, J. M., Snyder, E. Y. & Arenas, E. Neuroprotection through delivery of glial cell line-derived neurotrophic factor by neural stem cells in a mouse model of Parkinson's disease. *J. Neurosci.* **21**, 8108–8118 (2001).
70. Casper, D. *et al.* Enhanced vascularization and survival of neural transplants with *ex vivo* angiogenic gene transfer. *Cell Transplant.* **11**, 331–349 (2002).
71. Yasuhara, T. *et al.* Neurorescue effects of VEGF on a rat model of Parkinson's disease. *Brain Res.* **1053**, 10–18 (2005).
72. Tuszynski, M. H. *et al.* A phase 1 clinical trial of nerve growth factor gene therapy for Alzheimer disease. *Nature Med.* **11**, 551–555 (2005).
73. Kaspar, B. K., Llado, J., Sherkat, N., Rothstein, J. D. & Gage, F. H. Retrograde viral delivery of IGF-1 prolongs survival in a mouse ALS model. *Science* **301**, 839–842 (2003).
74. Azzouz, M. *et al.* VEGF delivery with retrogradely transported lentivector prolongs survival in a mouse ALS model. *Nature* **429**, 413–417 (2004).
75. Klein, S. M. *et al.* GDNF delivery using human neural progenitor cells in a rat model of ALS. *Hum. Gene Ther.* **16**, 509–521 (2005).
76. Ehtesham, M. *et al.* Induction of glioblastoma apoptosis using neural stem cell-mediated delivery of tumor necrosis factor-related apoptosis-inducing ligand. *Cancer Res.* **62**, 7170–7174 (2002).
77. Kim, S. K. *et al.* PEX-producing human neural stem cells inhibit tumor growth in a mouse glioma model. *Clin. Cancer Res.* **11**, 5965–5970 (2005).
78. Snyder, E. Y., Taylor, R. M. & Wolfe, J. H. Neural progenitor cell engraftment corrects lysosomal storage throughout the MPS VII mouse brain. *Nature* **374**, 367–370 (1995).
79. Monje, M. L. & Palmer, T. Prevention of deficits in neurogenesis with anti-inflammatory agents. US Patent application 20040254152 (2004).
80. Hains, B. C., Johnson, K. M., Eaton, M. J., Willis, W. D. & Hulsebosch, C. E. Serotonergic neural precursor cell grafts attenuate bilateral hyperexcitability of dorsal horn neurons after spinal hemisection in rat. *Neuroscience* **116**, 1097–1110 (2003).
81. Hofstetter, C. P. *et al.* Allodynia limits the usefulness of intraspinal neural stem cell grafts; directed differentiation improves outcome. *Nature Neurosci.* **8**, 346–353 (2005).
82. Weissleder, R. & Ntziachristos, V. Shedding light onto live molecular targets. *Nature Med.* **9**, 123–128 (2003).
83. Allport, J. R., Shinde Patil, V. R. & Weissleder, R. Murine neuronal progenitor cells are preferentially recruited to tumor vasculature via $\alpha_4$-integrin and SDF-1α-dependent mechanisms. *Cancer Biol. Ther.* **3**, 838–844 (2004).
84. Burnstein, R. M. *et al.* Differentiation and migration of long term expanded human neural progenitors in a partial lesion model of Parkinson's disease. *Int. J. Biochem. Cell Biol.* **36**, 702–713 (2004).
85. Ahn, T. B., Kim, J. M., Kwon, K. M., Lee, S. H. & Jeon, B. S. Survival and migration of transplanted neural stem cell-derived dopamine cells in the brain of parkinsonian rat. *Int. J. Neurosci.* **114**, 575–585 (2004).
86. Chen, J., Magavi, S. S. & Macklis, J. D. Neurogenesis of corticospinal motor neurons extending spinal projections in adult mice. *Proc. Natl Acad. Sci. USA* **101**, 16357–16362 (2004).
87. Shihabuddin, L. S. *et al.* Intracerebral transplantation of adult mouse neural progenitor cells into the Niemann–Pick-A mouse leads to a marked decrease in lysosomal storage pathology. *J. Neurosci.* **24**, 10642–10651 (2004).
88. Hayashi, T. *et al.* Neural precursor cells division and migration in neonatal rat brain after ischemic/hypoxic injury. *Brain Res.* **1038**, 41–49 (2005).
89. Shear, D. A. *et al.* Neural progenitor cell transplants promote long-term functional recovery after traumatic brain injury. *Brain Res.* **1026**, 11–22 (2004).
90. Wennersten, A., Meier, X., Holmin, S., Wahlberg, L. & Mathiesen, T. Proliferation, migration, and differentiation of human neural stem/progenitor cells after transplantation into a rat model of traumatic brain injury. *J. Neurosurg.* **100**, 88–96 (2004).
91. Picard-Riera, N. *et al.* Experimental autoimmune encephalomyelitis mobilizes neural progenitors from the subventricular zone to undergo oligodendrogenesis in adult mice. *Proc. Natl Acad. Sci. USA* **99**, 13211–13216 (2002).

92. Ben-Hur, T. *et al.* Transplanted multipotential neural precursor cells migrate into the inflamed white matter in response to experimental autoimmune encephalomyelitis. *Glia* **41**, 73–80 (2003).
93. Totoiu, M. O., Nistor, G. I., Lane, T. E. & Keirstead, H. S. Remyelination, axonal sparing, and locomotor recovery following transplantation of glial-committed progenitor cells into the MHV model of multiple sclerosis. *Exp. Neurol.* **187**, 254–265 (2004).
94. Ji, J. F., He, B. P., Dheen, S. T. & Tay, S. S. Expression of chemokine receptors CXCR4, CCR2, CCR5 and $CX_3CR1$ in neural progenitor cells isolated from the subventricular zone of the adult rat brain. *Neurosci. Lett.* **355**, 236–240 (2004).
95. Krathwohl, M. D. & Kaiser, J. L. Chemokines promote quiescence and survival of human neural progenitor cells. *Stem Cells* **22**, 109–118 (2004).
96. Martinez-Serrano, A. & Bjorklund, A. *Ex vivo* nerve growth factor gene transfer to the basal forebrain in presymptomatic middle-aged rats prevents the development of cholinergic neuron atrophy and cognitive impairment during aging. *Proc. Natl Acad. Sci. USA* **95**, 1858–1863 (1998).
97. Sun, L., Lee, J. & Fine, H. A. Neuronally expressed stem cell factor induces neural stem cell migration to areas of brain injury. *J. Clin. Invest.* **113**, 1364–1374 (2004).
98. Jin, K., Mao, X. O., Sun, Y., Xie, L. & Greenberg, D. A. Stem cell factor stimulates neurogenesis *in vitro* and *in vivo*. *J. Clin. Invest.* **110**, 311–319 (2002).
99. Schanzer, A. *et al.* Direct stimulation of adult neural stem cells *in vitro* and neurogenesis *in vivo* by vascular endothelial growth factor. *Brain Pathol.* **14**, 237–248 (2004).
100. Martinez-Serrano, A., Hantzopoulos, P. A. & Bjorklund, A. *Ex vivo* gene transfer of brain-derived neurotrophic factor to the intact rat forebrain: neurotrophic effects on cholinergic neurons. *Eur. J. Neurosci.* **8**, 727–735 (1996).
101. Low, W. C. *et al.* Function recovery following neural transplantation of embryonic septal nuclei in adult rats with septohippocampal lesions. *Nature* **300**, 260–262 (1982).
102. Ehtesham, M. *et al.* The use of interleukin 12-secreting neural stem cells for the treatment of intracranial glioma. *Cancer Res.* **62**, 5657–5663 (2002).
103. Barresi, V. *et al.* Transplantation of prodrug-converting neural progenitor cells for brain tumor therapy. *Cancer Gene Ther.* **10**, 396–402 (2003).
104. Lee, J. *et al.* Cellular and genetic characterization of human adult bone marrow-derived neural stem-like cells: a potential antiglioma cellular vector. *Cancer Res.* **63**, 8877–8889 (2003).
105. Shah, K. *et al.* Glioma therapy and real-time imaging of neural precursor cell migration and tumor regression. *Ann. Neurol.* **57**, 34–41 (2005).
106. Eaton, M. J., Santiago, D. I., Dancausse, H. A. & Whittemore, S. R. Lumbar transplants of immortalized serotonergic neurons alleviate chronic neuropathic pain. *Pain* **72**, 59–69 (1997).
107. Eaton, M. J., Plunkett, J. A., Karmally, S., Martinez, M. A. & Montanez, K. Changes in GAD- and GABA-immunoreactivity in the spinal dorsal horn after peripheral nerve injury and promotion of recovery by lumbar transplant of immortalized serotonergic precursors. *J. Chem. Neuroanat.* **16**, 57–72 (1998).
108. Eaton, M. J. *et al.* Transplants of neuronal cells bioengineered to synthesize GABA alleviate chronic neuropathic pain. *Cell Transplant.* **8**, 87–101 (1999).
109. Eaton, M. J. *et al.* Lumbar transplant of neurons genetically modified to secrete galanin reverse pain-like behaviors after partial sciatic nerve injury. *J. Peripher. Nerv. Syst.* **4**, 245–257 (1999).
110. Cejas, P. J. *et al.* Lumbar transplant of neurons genetically modified to secrete brain-derived neurotrophic factor attenuates allodynia and hyperalgesia after sciatic nerve constriction. *Pain* **86**, 195–210 (2000).
111. Lin, C. R. *et al.* Intrathecal spinal progenitor cell transplantation for the treatment of neuropathic pain. *Cell Transplant.* **11**, 17–24 (2002).

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## Competing interests statement
The authors declare no competing financial interests.

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