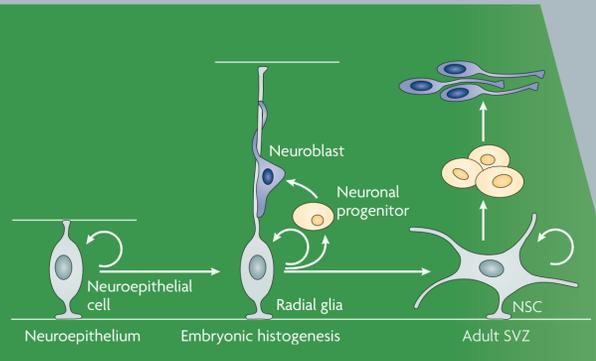


Neural stem cells are capable of self-renewal and can generate neurons, astrocytes and oligodendrocytes. During nervous system development, NSCs within the primitive neural ectoderm give rise to neural progenitors, which rapidly become regionally and temporally specified, first generating large projection neurons and later small interneurons and glia. Small numbers of NSCs persist in the adult brain. They proliferate slowly and produce new neurons throughout life to replenish cells in the

hippocampus and olfactory bulb. NSCs and progenitor cells can be isolated from embryonic stem cells, induced pluripotent stem cells, and fetal and adult brain samples. They can be induced to differentiate into neurons and glia *in vitro* and *in vivo*. NSCs grown in culture allow *in vitro* modeling of nervous system development and diseases. NSCs are also under investigation as potential therapeutic agents for neurodegenerative diseases and nervous system injury.

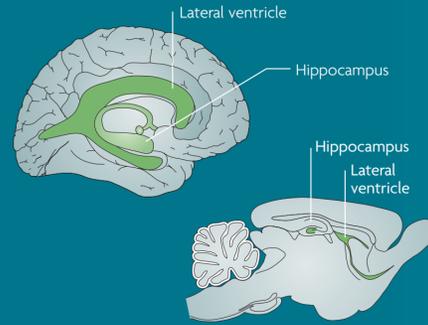
Fetal neural stem cells

The CNS begins as a tube of neuroepithelial cells, the most primitive form of neural stem cells. In the cortex, neuroepithelial cells transition into radial glial cells, which then give rise to neural progenitors, neurons, astrocytes and oligodendrocytes. In other regions of the developing CNS¹ such as spinal cord and striatum, radial glial cells are not as prominent, and progenitors emerge from nonradial multipotent NSC populations. True NSCs are difficult to expand from fetal brain tissue. They may be better thought of as regionally pre-specified progenitor cells with characteristics of the region from which they were initially isolated.



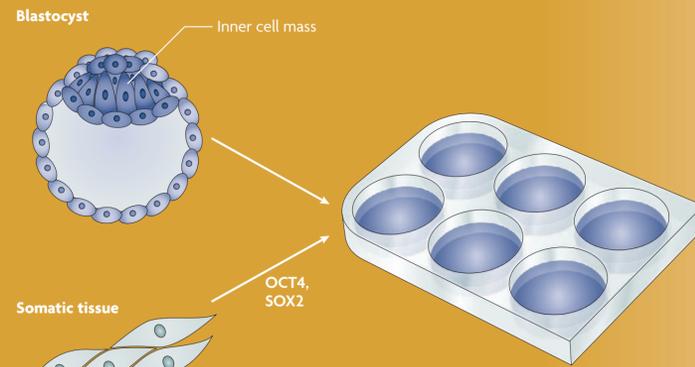
Adult neural stem cells

NSCs are located within two regions of the adult human and rodent brain (green): the subgranular zone of the hippocampus and the subventricular zone of the striatum². Adult NSCs generate new neurons throughout life that integrate into hippocampal and olfactory circuits and are thought to be important for memory and olfaction. These NSCs can be isolated and expanded from rodent brains; however, they are more difficult to isolate from human brain biopsies or autopsy samples. Another type of NSC outside these two regions expresses the marker NG2 and can also proliferate *in vitro* and *in vivo*. However, this cell type does not normally give rise to new neurons *in vivo*. NG2 cells can be activated after injury and can generate new oligodendrocytes.



Early embryo

The inner cell mass from the blastocyst stage of the early embryo contains ES cells. These are truly pluripotent stem cells that can generate any tissue of the body, including NSCs. The derivation of human ES cells, however, has been very controversial for ethical reasons, prompting the search for alternative NSC sources.



Adult somatic cells

Adult differentiated cells are unipotent, able to generate only their own kind. Adult fibroblasts, which can be obtained easily from skin biopsies, as well as adult cells from other sources, can be converted into iPS cells by overexpression of a few genes, including the transcription factors OCT4 and SOX2. Adult cells from other sources have also been converted to iPS cells. iPS cells are similar to ES cells in many ways, and can be differentiated into NSCs *in vitro* using similar techniques.

ES and iPS cells

Pluripotent ES and iPS cells can be expanded indefinitely in culture because they express telomerase, which prevents chromosome aging. Both stem cell types are able to make any tissue in the body, including NSCs.

Derivation and expansion of NSCs

To convert different tissue sources into NSCs, cells derived from fetal (green box), adult (teal box) or ES/iPS (gold box) sources are cultured in media containing the mitogens EGF and FGF-2. NSCs derived from these sources can be expanded either as spherical aggregates termed 'neurospheres' or as monolayer 2D cultures. They can turn into neurons, astrocytes and oligodendrocytes, depending on the growth and differentiation factors they are exposed to during subsequent *in vitro* differentiation steps.

Differentiation

To promote NSC differentiation, EGF and FGF-2 are usually replaced with specific morphogens or growth factors that promote initial maturation into either neurons or glia. Final differentiation into specific neuron and glial types requires other morphogens and growth factors, and in some cases transcription factors. Current protocols for differentiation of specific neuron and glial types are indicated along the shaded paths. Yield varies substantially for the different cell types. In some cases only NSCs from certain sources can generate specific types of neural tissue. For example, only ES and iPS derived NSCs (gold shaded lines) can generate all types of neurons, whereas fetal and adult-derived NSCs do not easily make dopaminergic and motor neurons after expansion in culture.

Transplantation

Expanded naive or partially differentiated populations of NSCs can be transplanted into the CNS of experimental animals to test their potential to differentiate into functional neuronal or glial cells *in vivo*. Such animal studies are the first steps towards developing cell therapies for neurological disorders. At present, two clinical trials are under way in the USA, transplanting fetally derived NSCs into the brains of children with Batten's disease and adults with ALS. Such transplants may help by replacing neural tissue and/or by releasing growth factors that support any remaining functional tissue. One of the major challenges with NSC-derived transplants is a chance of tumor growth from residual mitogenic cells. ES cell derived transplants in particular may grow teratomas from residual pluripotent, dividing cells. Fetal- or adult-brain-derived transplants cannot grow teratomas but still carry some risk of tumorigenicity, which will require careful monitoring. Immune rejection issues will also be a challenge for this field.

Abbreviations

ALS, amyotrophic lateral sclerosis; BDNF, brain-derived neurotrophic factor; BMP, bone morphogenetic factor; cAMP, cyclic adenosine monophosphate; CNS, central nervous system; CNTF, ciliary neurotrophic factor; Dkk1, Dickkopf-1; EGF, epidermal growth factor; ES, embryonic stem (cell); FDA, US Food and Drug Administration; FGF, fibroblast growth factor; GABA, γ -aminobutyric acid; iPS, induced pluripotent stem (cell); LIF, leukemia inhibitory factor; NG2, nerve/glia antigen 2; NGN, neurogenin; NSC, neural stem cell; NT, neurotrophin; PDGF, platelet-derived growth factor;

PMN, Purmorphamine; RA, retinoic acid; Shh, Sonic hedgehog; SVZ, subventricular zone; VN, vitronectin; 2D, two-dimensional

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Huntington's disease

Loss of inhibitory GABAergic neurons in the striatum leads to uncontrolled movement in Huntington's disease patients. As the disease progresses other brain areas degenerate, and patients suffer severe cognitive decline. Replacement or protection of striatal GABAergic neurons using NSC-derived cells may slow the disease, but the progressive spread of degeneration is difficult to address. Grafting of NSCs into multiple sites in both the striatum and cortex might be a feasible approach.

Parkinson's disease

Parkinson's disease is caused by loss of dopamine production in the brain's basal ganglia. Dopamine-producing neurons can be derived from ES cells and could be used eventually to replace those that are lost to disease. Although this idea works in animal models, human trials using primary fetal tissue revealed that new dopamine neurons could cause side effects. More work in animal models will be necessary to uncover mechanisms that may enable the functional integration of transplanted dopaminergic neurons.

Amyotrophic lateral sclerosis

In ALS, progressive paralysis is caused by loss of cortical and spinal motor neurons. Efforts to replenish motor neurons from NSC transplants are challenging, as the new neurons would need to grow axons over long distances to connect to the denervated muscles. An alternative approach to cell therapy for ALS is the transplantation of NSC-derived astrocytes to protect remaining motor neurons in early-stage patients from further degeneration.

Spinal cord injury

Trauma to the spinal cord results in paralysis below the site of injury due to severed long-distance connections between the brain and limb muscles. An FDA-approved protocol uses human ES cell derived NSCs to generate new oligodendrocytes in the injured spinal cord, expecting that remyelination of remaining damaged but not severed axons would increase their conductance and improve function.

Multiple sclerosis

Oligodendrocyte degeneration leads to demyelination of axons, which causes slowed conductance leading to a multitude of neurological symptoms. In promising animal studies, transplantation of pre-differentiated NSCs results in remyelination and renewed motor function. In human patients, the challenge is to target transplanted cells to the many demyelinated lesions that are widely dispersed throughout the CNS.

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