Neural stem cells: therapeutic potential for neurodegenerative diseases

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Introduction: Neural stem cells (NSCs) from specific brain areas or developed from progenitors of different sources are of therapeutic potential for neurodegenerative diseases.

Sources of data: Treatment strategies involve the (i) transplantation of exogenous NSCs; (ii) pharmacological modulations of endogenous NSCs and (iii) modulation of endogenous NSCs via the transplantation of exogenous NSCs.

Areas of agreement: There is a consensus about the therapeutic potential of transplanted NSCs. The ability of NSCs to home into areas of central nervous system injury allows their delivery by intravenous injection. There is also a general agreement about the neuroprotective mechanisms of NSCs involving a ‘bystander effect’.

Areas of controversy: Individual laboratories may be using phenotypically diverse NSCs, since these cells have been differentiated by a variety of neurotrophins and/or cultured on different ECM proteins, therefore differing in the expression of neuronal markers.

Growing points: Optimization of the dose, delivery route, timing of administration of NSCs, their interactions with the immune system and combination therapies in conjunction with tissue engineered neural prostheses are under investigation.

Areas timely for developing research: In-depth understanding of the biological properties of NSCs, including mechanisms of therapy, safety, efficacy and elimination from the organism. These areas are central for further use in cell therapy.

Cautionary note: As long as critical safety and efficacy issues are not resolved, we need to be careful in translating NSC therapy from animal models to patients.

Keywords: neural stem cells/neurodegeneration/neuroprotection/transplantation/modulation/neurogenesis/anti-inflammatory/bystander effect/angiogenesis

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Neural stem cells (NSCs) are multipotent, tissue-resident, lineage-restricted neural progenitor cells that are capable of self-renewal as long as they remain undifferentiated. By definition, NSCs have the potential, upon appropriate differentiation steps, to generate the main phenotypes of the nervous system, i.e. neurons, astrocytes and oligodendrocytes. NSCs are present in the developing and adult central nervous system (CNS) of mammals, specifically in neurogenic areas of the brain, such as the subventricular (SVZ) and subgranular zone (SGZ). The neurogenic niche represents a unique micro-environment that determines the survival and fate of NSCs by paracrine effects, such as heterotypic cell–cell contacts and interactions with specific extracellular matrix (ECM) components. The identification of undifferentiated NSCs in situ is controversial in the absence of a set of established phenotypic markers. As of today, NSCs are generally identified by their ability for self-renewal, CNS-phenotypic differentiation and the appearance of neuronal electrogenic machinery. Some level of consensus exists in terms of characterization of differentiating or differentiated cells based on a combination of established ‘markers for mature phenotypes’, such as nestin, β-tubulin III, microtubule-associated protein 2 (MAP2), neuronal nuclei (NeuN) and glial fibrillary acid protein (GFAP).

Neurodegenerative diseases (NDs) share common mechanisms leading to neuronal dysfunction and, eventually, cell death. Current therapies address the symptoms, but are yet inefficient for rescuing or regenerating cellular function or even halting the neuronal death process. There are three independent, ‘rate-limiting’ processes, each of which may be the bottleneck for successful cell-based therapies: (i) survival of the transplanted/injected cells, (ii) targeting of the cells to the injured area and (iii) integrating the cells into the host’s neuronal circuits. Transplanted NSCs are able to migrate and specifically home into the sites of injury. These cells are, therefore, of potential therapeutic value in the treatment of diverse neuronal disorders. The proof-of-concept for the therapeutic potential of NSCs has been demonstrated in vitro and also in vivo in select animal models of different NDs, such as Parkinson’s (PD), Huntington’s, Alzheimer’s (AD), diseases, multiple sclerosis, Amyotrophic lateral sclerosis (ALS), experimental autoimmune encephalomyelitis, spinal cord injury (SCI), stroke and traumatic brain injury (TBI). Based on these initial proof-of-concept studies ascertaining the therapeutic potential of NSCs, a few clinical trials have been initiated to evaluate safety (Phase I) and efficacy (Phase II, III) of NSC treatment for advanced PD, ALS, stroke and chronic SCI. The results of the majority
of these clinical studies have not yet been reported; however, preliminary data from some limited studies indicate safety and trends of efficacy.\textsuperscript{8–11} In terms of neuropharmacological mechanisms, the above-mentioned beneficial therapeutic effects of stem cells (SCs) are mediated by specific processes that involve neuroprotection and/or neurogenesis.\textsuperscript{5,6} In general terms, neuroprotection refers to the activation of biochemical and cellular pathways that antagonize neuronal cell death. Neurogenesis is the process of proliferation of endogenous neural stem/progenitor cells, yielding post-mitotic neurons. Neurogenesis is controlled by a variety of molecules, such as pro- and anti-inflammatory cytokines (e.g. interferon gamma, tumor necrosis factor alpha, interleukin-1 beta (IL-1\beta), IL-6 as pro-inflammatory,\textsuperscript{6,12} and IL-4, transforming growth factor (TGF) beta as anti-inflammatory cytokines\textsuperscript{2,4}), neurotransmitters (including gamma-aminobutyric acid, dopamine, glutamate, acetylcholine, serotonin, norepinephrine)\textsuperscript{5} and oxidative stress-related radicals (such as nitric oxide).\textsuperscript{5,12} Neurogenesis is also initiated by activation of different signaling pathways, such as MAPK, CXCR4 and NF-\kappaB,\textsuperscript{12} which are commonly found during embryonic development and are generated or activated in the wake of NDs and brain injury. One of the confounding complications in utilizing NSCs as exogenous sources for neurogenesis is the fact that different cell populations used in the literature may have vastly different differentiation potential. They differentiate into all kinds of CNS cells, as mentioned above, or they can be lineage restricted and only differentiate into bona fide neurons.\textsuperscript{13,14} On the other hand, there is apparently no significant difference in the neuroprotective competence of differentiated versus undifferentiated cells.\textsuperscript{15}

The injured brain responds to the insult by activating a variety of defense mechanisms aimed at repairing neuronal damage.\textsuperscript{2,3} These mechanisms include enhanced synthesis and secretion of neurotrophins (the first line of defense), as well as release from microglia and astrocytes of pro- and anti-inflammatory molecules, which are the main mediators of inflammation in the brain parenchyma.\textsuperscript{16} These initial signals induce cell proliferation\textsuperscript{3} and trigger the mobilization of SVZ- and/or SGZ- resident endogenous NSCs and their migration to the site of injury in order to assist in neuroprotection and in the long-term regeneration of neuronal pathways.\textsuperscript{4,5} Secondary to these initial events, inflammatory cells from the periphery invade the brain to also assist in tissue repair and to provide a balance between pro- and anti-inflammatory cytokine secretion.\textsuperscript{12} Neurodegenerative processes can promote neurogenesis and cause the migration, differentiation and integration of these SVZ- and SGZ-resident NSCs into functional neuronal circuits.\textsuperscript{3} In mild insults, these endogenous mechanisms of repair and regeneration may be sufficient. However, with severe injuries the
endogenous attempts at repairing and restoring neuron activity fail to provide neuroprotection. Therefore, endogenous repair alone is, in general, not enough for functional recovery. Supplementation of these processes by transplantation of NSCs may in some cases alleviate this shortcoming.

Current regenerative strategies for treating NDs consider three distinct approaches: (i) introduction of SCs, isolated from various origins, that can target the injured area in the CNS upon i.v. or local transplantation; (ii) pharmacological interventions to modulate proliferation, migration and differentiation of endogenous NSCs to increase their efficacy in neurogenic areas of the brain, such as SVZ and SGZ and (iii) combined approaches to obtain additive and synergistic effects by modulation of endogenous NSCs via transplantation of exogenous NSCs.

Regenerative strategies for treating NDs

NSCs’ transplantation-based therapy

Given their therapeutic potential in different NDs, the transplantation of NSCs is a promising approach. NSC progenitors of fetal origin can be isolated from bone marrow, umbilical cord blood and fetal brain. The mechanisms by which the transplanted NSCs exert their neuroprotective effects have yet to be fully elucidated; however, several trends have been established:

(i) Some of the transplanted NSCs proliferate, migrate and differentiate although in very small numbers and in some cases lead to functional recovery (Table 1). However, it is not yet clear, how the transplanted cells are homing to the injured area, although the migration on meninges and choroid plexus is involved, e.g. by the expression and/or secretion of chemo-attractants or chemo-repellers, which direct SCs migration. It is difficult to generate in vivo a relationship between the number of cell transplanted and the efficacy of regeneration process, which may differ depending on the kind and the size of the lesion as well as its specific location in the brain. Each disorder, whether stroke, TBI, AD or PD, has its own time course and cannot be fully mimicked in animal models and the clinical trials with mesenchymal stem cells have not yet addressed the issue. Hence, it is difficult to correlate therapeutic efficacy of the transplanted cells to the ‘loudness’ of the degenerative signal.

(ii) Being resistant to hypoxic conditions, some of the transplanted cells will survive for a limited period of time in an hostile environment...
### Table 1 Emerging therapeutic strategies of NDs using stem cells.

#### Neuroprotective mechanism

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<tr>
<th>Therapeutic strategy</th>
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<td>Transplantation of exogenous NSCs</td>
<td>PD 6-OHDA 6-OHDA</td>
<td>i.n. drops</td>
<td>Rats GFP BM-derived MSCs CD9+, CD29+, CD44+, CD73+, CD90+, CD166+, CD200+, SSEA1+ and CD11b/ c−, CD31−, CD34−, CD39−, CD45−, CD106−, CD133−, CD143−</td>
<td>β-tubulin III, TH, PNCA</td>
<td>BDNF</td>
<td>ni</td>
<td>Decreased IL-1β, IL-2, -6, -12, TNFα, IFN-γ and GM-CSF</td>
<td>ni</td>
<td>SMAα</td>
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<td></td>
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<td>i.s.</td>
<td>Human adult MSCs</td>
<td>Differentiation towards GFAP-positive glial phenotype Increased synaptobrevin expression</td>
<td>MAP2, GFAP, IB4</td>
<td>BDNF, GDNF</td>
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<td>Stroke MCAO WT and tPA-/-mice</td>
<td>ALS SOD1 G93A transgenic rats</td>
<td>i.c. spinal cord into the ventral horn</td>
<td>GFP GRPs derived from developing spinal cord</td>
<td>GLT1, GFAP, nestin, synapsin</td>
<td>ni</td>
<td>Neuronal outgrowth, synaptogenesis Neurite remodeling BMP2, -4</td>
<td>Increased SVZ VEGF, HGF, MMP9</td>
<td>ni</td>
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<td>Modulation of endogenous NSCs</td>
<td>PD 6-OHDA</td>
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<td>Stroke MCAO</td>
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<td></td>
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<td>ni</td>
<td>Increased SVZ VEGF, angiopeotin</td>
<td>ni</td>
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<td>Stroke MCAO WT and tPA-/-mice</td>
<td>i.n. or i.v. tail vein infusion of tPA and Niaspan</td>
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<td>AD</td>
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<td>Modulation of endogenous NSCs via transplantation of exogenous NSCs</td>
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<td>ni</td>
<td>ni</td>
<td>Increased SVZ</td>
<td>Yasuhara et al.46</td>
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<td>Stroke</td>
<td>MCAO</td>
<td>Ipsilateral to the ischemic lesion</td>
<td>Fik human BM-derived MSCs CD29+, CD44+, CD105+, and CD31+, CD34−, CD45−, HLA-DR−</td>
<td>Neuronal markers musashi1, doublecortin, NeuN, synaptophysin, GlT1, c-fos</td>
<td>BDNF, TGF</td>
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<td>ni</td>
<td>Increased SVZ</td>
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<td>SCI dorsal column lesion at C1</td>
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<td>EAE</td>
<td>s.c.</td>
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<td>EAE</td>
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<td>Mice GFP BM-derived MSCs CD9+, CD44+, CD73+, Sca1+ and CD11b−, CD34−, CD45− or embryonal neurospheres</td>
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<td>ni</td>
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<td>Zappia et al.49</td>
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NSCs, neural stem cells; PD, Parkinson’s disease; 6-OHDA, 6-hydroxydopamine; MCAO, middle cerebral artery occlusion; tPA, tissue plasminogen activator; ALS, amyotrophic lateral sclerosis; SOD1, Cu/Zn superoxide dismutase 1; AD, Alzheimer’s disease; TBI, traumatic brain injury; SCI, spinal cord injury; EAE, experimental autoimmune encephalomyelitis; MOG35-55, encephalitogenic peptide for EAE induction; i.n., intranasal; i.s., intrastriatal; i.v., intravenous; i.c., intracervical; i.v.c., intraventricular; TGfα, transforming growth factor alpha; PDEI, phosphodiesterase inhibitor; NGF, nerve growth factor; bFGF, basic fibroblast growth factor; VEGF, vascular endothelial growth factor; s.c., subcutaneous; GFP, green fluorescent protein; BM, bone marrow; MSCs, mesenchymal stem cells; PNPCs, porcine expanded neural precursor cells; GRPs, glial-restricted precursors; ni, not relevant; SVZ, subventricular zone; TH, tyrosine hydroxylase; PNCA, proliferating cell nuclear antigen; GFAP, glial fibrillary acidic protein; MAP2, microtubule-associated protein 2; IB4, lectin, neuronal marker; GLT1, glutamate transporter 1; NeuN, neuronal nuclei; NCAM, neuronal cell adhesion molecule; ni, not investigated; GaIC, galacitcerebroside C, oligodendrocytes marker; BDNF, brain-derived neurotrophic factor; GDNF, glial cell line-derived neurotrophic factor; IL, interleukin; TNFα, tumor necrosis factor alpha; IFNγ, interferon gamma; GM-CSF, granulocyte-macrophage colony-stimulating factor; BMP2,4, bone morphogenetic protein 2 or 4; DCs, dendritic cells; SHH, sonic hedgehog; GZ, granule zone; SAMA, smooth muscle actin alpha; HGF, hepatocyte growth factor; MMP9, matrix metalloproteinase 9; vWF; Von Willebrand factor.
in the injured brain,\textsuperscript{6} and are therefore able to exert their beneficial trophic effects.

(iii) Some of transplanted NSCs enter into an ‘internal crosstalk’, in that the immature NSCs provide a protective environment for their daughter cells, that will undergo neuronal differentiation, by either secreting neuroprotective/neurotrophic factors,\textsuperscript{2,4–6} such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF) and neurotrophin-3\textsuperscript{2,5} and/or by modulating the host immune response\textsuperscript{4} (Table 1).

(iv) The greatest beneficiaries from this kind of protective support are both endogenous NSCs that are mobilized to migrate to and integrate into the site of injury as well as injured and/or normal neurons on the penumbra of the injured site, which are more protected.\textsuperscript{6}

Additionally, the local reactive astrocytes may also serve as mediators of neuroprotection, to promote the survival of limited numbers of exogenous progenitors transplanted in the site of brain injury and to facilitate their robust functional activity, as inferred from the release of neurotrophic factors, such as GDNF,\textsuperscript{22} BDNF, NGF, bFGF, vascular endothelial growth factor (VEGF), etc. (Table 1). An important conclusion from the majority of pre-clinical studies is that most of the putative therapeutic benefits from NSC transplantation may actually stem from the fact that NSCs exert beneficial ‘bystander effects’ to the micro-environment.\textsuperscript{2} For example, Kranz and colleagues\textsuperscript{13} showed that administration of placenta derived mesenchymal stem cells (MSCs) lead to behavioral recovery and reduction in lesion size after stroke by peripheral immune suppression and reactivation of glial cells. Some studies indicate that NSCs might integrate into neuronal pathways and functionally regenerate the damaged brain tissue. For example, Bjorklund \textit{et al.}\textsuperscript{23} using positron emission tomography (PET) scanning reported that transplanted embryonic SCs (ESCs) developed spontaneously into dopaminergic neurons. Such dopaminergic neurons restored cerebral function and behavior in an animal model of Parkinson’s disease. Using an elegant optogenetic targeting approach, Weick \textit{et al.}\textsuperscript{24} demonstrated that transplanted human ESCs differentiate into neurons and that, upon optical excitation, these neurons function and integrate in the context of host brain circuitry, supporting the notion of a polysynaptic pathway integration of transplanted SCs. Finally, studies by Denham \textit{et al.}\textsuperscript{25} suggest that GFP\textsuperscript{+} human pluripotent ESCs have the capacity for directed differentiation into a variety of neuronal subtypes, which, upon transplantation, extend long-distance axonal projections that will grow along host white matter tracts.

One of the disadvantages of the transplanted NSCs is their limited survival \textit{in situ} (both in terms of numbers and duration), which might explain the insufficient therapeutic efficacy and prevent, in a simplistic
way, the translation of pre-clinical data to clinical trials.\textsuperscript{2,5,6} A potential approach to overcoming this problem is the manipulation of the environment to make it more hospitable using tissue engineering approaches. For example, the incorporation of the transplanted NSCs into injectable polymeric scaffolds can improve their survival by providing physical protection against acute or chronic inflammatory responses.\textsuperscript{26–28} Furthermore, these scaffolds can be modified to release neurotrophins in a controlled fashion, providing trophic support to the NSCs in the scaffold as well as to the endogenous brain parenchyma.\textsuperscript{29}

**Modulation of endogenous NSCs**

The ability of the precursor cell to either proliferate or differentiate is dependent on intrinsic and environmental stem-cell niche-derived features.\textsuperscript{3,5} Both niches (SVZ and SGZ) are enriched in axons that secrete a variety of neurotransmitters, which in turn play an important role in the regulation of neurogenesis in these niches.\textsuperscript{5} Several groups succeeded in stimulating endogenous neurogenesis and NSCs differentiation by administering certain neurotrophins and other growth factors, as well as specific chemo-attractants (Table 1). For example, in the 6-hydroxydopamine (6-OHDA) PD rat model, TGF alpha (TGF\(\alpha\)) induced massive proliferation and substantial migratory waves of nestin-positive neuronal progenitor cells from the SVZ to the injured site. Therefore, the intrastriatal TGF\(\alpha\) infusion might provide a feasible exogenous intervention mechanism that will likely induce NSCs proliferation within the SVZ with the aim to restore the neuro-ectoderm-like properties of the regenerating tissue (Table 1).

Although the stimulation of endogenous NSCs seems to be a promising technique, studies in animal models (Table 1) have as yet failed to demonstrate a significant therapeutic outcome.\textsuperscript{5} In order to advance this approach as a promising therapy for NDs it will be important to develop novel technologies for the delivery of growth factors across the blood–brain barrier and to identify critical micro-environmental cues involved in the regulation of the proliferation, migration and differentiation of SVZ/SGZ progenitors.

**Combined approach of modulating endogenous NSCs via transplanting NSCs**

The currently accepted theory regarding the interactions between exogenous and endogenous NSCs relies on two mechanisms: (i) secretion of neurotrophins, cytokines and chemokines and (ii) modulation of the immune system, particularly inflammation.\textsuperscript{4,5}
The interaction between implanted SCs and the endogenous immune-inflammatory system affects the endogenous NSCs and can provide either a hostile or a permissive environment for neurogenesis, depending on the context.\textsuperscript{4} The immune response following SCs transplantation is controversial.\textsuperscript{1,2} Some studies found that anti-inflammatory cytokines have a positive effect on neurogenesis, while pro-inflammatory molecules inhibit or thwart proper neurogenesis.\textsuperscript{2,4,6} However, other studies reported that the very same pro-inflammatory cytokines can initiate a neurogenic response\textsuperscript{2,3} (Table 1).

Collectively, the findings summarized in Table 1 indicate that a variety of distinct SCs from different tissue sources, specifically MSCs, are able to induce anti-inflammatory and immunosuppressive effects in animal models of neurological disorders, in which typically inflammation is an integral component. This property of the transplanted SCs to modulate inflammation, stimulate neurogenesis and promote migration to the site of lesion will create an environment more suitable for neuroprotection and repair.

**Conclusions**

Exogenous SCs transplantation may provide the cues/signals necessary and/or sufficient for endogenous neural progenitor stimulation, indicating a synergy between exogenous and endogenous NSCs activities, which could be exploited for therapeutic purposes. The transplanted NSCs can provide neuroprotection by secreting trophic factors and also by immune modulation. In addition, they may cooperate with other cells in the brain to generate permissive niches for the activation of endogenous NSCs. Taken together the various modes of action might contribute to an enhanced neuroregenerative capacity of the host brain (Fig. 1). Understanding the specifics of the micro-environmental cues and signals in the stem cell niche, which foster the interaction between exogenous and endogenous SCs after transplantation, will provide novel targets that might be exploited for the therapy of neurodegenerative brain disorders. Therapies that combine the transplantation into the damaged brain of a sufficiently large number of NSCs to affect endogenous neurogenesis and injection of neurotrophic/neuromodulatory growth factors may provide the ideal approach for the therapy of NDs.

**Future challenges**

Survival and engraftment are two of the main obstacles to fully exploiting the therapeutic potential of the transplanted NSCs. Using tissue
Fig. 1 Scheme of mechanistic processes involved in NSCs-induced neuroprotection in NDs (PD, Parkinson’s disease; ALS, amyotrophic lateral sclerosis; AD, Alzheimer’s disease; TBI, traumatic brain injury; SCI, spinal cord injury; EAE, experimental autoimmune encephalomyelitis). Transplantation of exogenous NSCs (green cells) by variant routes of delivery (syringe; i.v.c., intraventricular; i.c., intracervical; i.s., intrastriatal; i.n., intranasal; i.v., intravenous) induced neuroprotection at the site of brain injury (lesion) by different mechanisms including the involvement of endogenous NSCs (blue cells) located at the SVZ. These mechanisms include bystander effects induced by exogenous and endogenous NSCs resulting in improved survival and/or neuroprotection of the insulted neuron (GF, growth factor). Alternatively, the direct effects may include the repair of neuronal circuits either by direct differentiation of the exogenous NSCs (repair 1) or endogenous NSCs (repair 2). The lack or presence of an action potential indicates damage or repair (respectively) in a neuronal pathway. In addition, the anti-inflammatory effects mediated by microglia (orange cells) or mobilization of peripheral hematopoietic cells (pink cells) may contribute to neuroprotection in the penumbra of the lesion site. Neurogenesis of either exogenous or endogenous NSCs or angiogenesis (capillary formation/penetration into the lesion area induced by angiogenic factors such as VEGF) may provide neuroprotective/neuroregenerative beneficial contributions.
engineering approaches can overcome this problem, e.g. by providing them a physical protection in injectable polymeric scaffolds, which will improve NSC survival. The race is on to find the ‘ultimate’ scaffold. It is also important to develop innovative technologies for efficiently delivering NSCs and/or neurotrophic growth factors across the blood–brain barrier.

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