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Neurorestorative Treatments for Traumatic Brain Injury

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Abstract

Traumatic brain injury (TBI) remains a major cause of death and permanent disability worldwide, especially in children and young adults. A total of 1.5 million people experience head trauma each year in the United States, with an annual economic cost exceeding $56 billion. Unfortunately, almost all Phase III TBI clinical trials have yet to yield a safe and effective neuroprotective treatment, raising questions regarding the use of neuroprotective strategies as the primary therapy for acute brain injuries. Recent preclinical data suggest that neurorestorative strategies that promote angiogenesis (formation of new blood vessels from pre-existing endothelial cells), axonal remodeling (axonal sprouting and pruning), neurogenesis (generation of new neurons) and synaptogenesis (formation of new synapses) provide promising opportunities for the treatment of TBI. This review discusses select cell-based and pharmacological therapies that activate and amplify these endogenous restorative brain plasticity processes to promote both repair and regeneration of injured brain tissue and functional recovery after TBI.

Keywords

angiogenesis; functional recovery; neurogenesis; plasticity; synaptogenesis; traumatic brain injury

Introduction

Traumatic brain injury (TBI) is a leading cause of mortality and morbidity worldwide, particularly among the young. Neuroprotection is an important strategy for the treatment of TBI (Narayan et al., 2002). To date, no effective neuroprotective agents have been identified from TBI clinical trials. The disappointing clinical trials may be due to variability in treatment approaches and heterogeneity of the population of TBI patients. Another important aspect is that most clinical trial strategies have used drugs that target a single pathophysiological mechanism, although many mechanisms are involved in secondary injury after TBI. Recent research has focused increasingly on multifunctional agents that target multiple injury mechanisms, particularly those that occur later after the insult (Stoica et al., 2009). Targeting multiple injury mechanisms that contribute to the secondary injury cascade may increase successful clinical trial outcomes.

Recent preclinical studies have revealed that TBI induces neurogenesis, axonal sprouting, and angiogenesis (Lu et al., 2004a; Lu et al., 2005; Oshima et al., 2009; Richardson et al.,

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2007; Xiong et al., 2010a; Zhang et al., 2010), which may contribute to the spontaneous functional recovery. Agents and treatments that promote these neurorestorative processes have been demonstrated to improve functional recovery after brain injury (Zhang and Chopp, 2009). However, clinical trials in TBI have primarily targeted neuroprotection, and trials directed specifically at neurorestoration have not been conducted. The promotion of neurorestorative processes may be a potential therapy for TBI. We review select cell-based and pharmacological therapies that enhance endogenous restorative brain plasticity processes to improve functional recovery after TBI.

Neurogenesis

Throughout life, neurogenesis occurs in all mammalian brains in the subventricular zone (SVZ) of the lateral ventricle and in the dentate gyrus subgranular zone (SGZ) of the hippocampus (Zhao et al., 2008). Newly generated neurons originate from neural stem cells (NSCs) in the adult brain. NSCs are self-renewing multipotent cells that generate glial and neuronal cells (Zhao et al., 2008). Granule neurons in the dentate gyrus of the hippocampus continuously die, and the neural stem/progenitor cells in the SGZ may proliferate to maintain a constant cell number in the dentate gyrus. Moreover, newly generated neurons in the dentate gyrus are capable of projecting axons into the CA3 region of the hippocampus in normal brains in rodents (Hastings and Gould, 1999). TBI induces hippocampal cell proliferation (Kernie et al., 2001; Lu et al., 2005; Xiong et al., 2008), and the vast majority of the newly generated cells in the SGZ that survive for 10 weeks after TBI differentiate into mature neurons (Sun et al., 2007). Newborn neurons extend axonal projections into the CA3 region as early as 2 weeks after TBI (Emery et al., 2005), which may contribute to cognitive recovery observed in rats that have experienced a TBI. In the normal adult brain, SVZ-derived neuroblasts migrate along the rostral migratory stream to the olfactory bulb, where these cells differentiate into interneurons to replace those that have died. After cortical injuries, a portion of neuroblasts generated in the SVZ migrate to injured areas instead of the rostral migratory stream. Following TBI, neuroblasts migrating from the SVZ can differentiate into neurons and glia (Kernie et al., 2001).

Angiogenesis

The adult central nervous system (CNS) vasculature is extremely stable under physiological conditions, but is activated after injury (Greenberg and Jin, 2005). Adult vascular remodeling includes angiogenesis by mature endothelial cells (that is, the formation of new capillaries from pre-existing vessels) and vasculogenesis (de novo formation of blood vessels when there are no pre-existing ones) by endothelial progenitor cells (EPCs). EPCs are present in the bone marrow and peripheral blood, and mobilize to the latter following TBI (Guo et al., 2009). There is a substantial increase in vasculogenesis following TBI (Morgan et al., 2007). Pharmacological agents such as erythropoietin (EPO) and statins increase the number, mobilization and functional activity of EPCs (Besler et al., 2008). EPO, statins, bone marrow stromal cells (MSCs), and thymosin beta4 promote angiogenesis and improve functional recovery in rats after TBI (Chopp and Li, 2002; Lu et al., 2004b; Lu et al., 2007b; Mammis et al., 2009; Wible and Laskowitz, 2010; Xiong et al., 2010a; Xiong et al., 2010b). The strategies for mobilization and/or transplantation of EPCs or treatment with angiogenesis-enhancing agents may emerge as promising approaches for the treatment of TBI.

Coupling of Neurogenesis and Angiogenesis

Neurovascular niches within the CNS consist of endothelial cells, pericytes, neurons and glial cells, as well as growth factors and extracellular matrix proteins surrounding the endothelium (Lok et al., 2007). The neurovascular niches provide microenvironments for

NSCs in the adult brain; newly generated, immature neurons are closely associated with the remodeling vasculature. The generation of new vasculature facilitates coupled neurorestorative processes including neurogenesis and synaptogenesis, which improve functional recovery (Li and Chopp, 2009; Zhang and Chopp, 2009). Angiogenesis and neurogenesis may play a significant role in mediating functional recovery following experimental TBI (Chopp et al., 2008; Li and Chopp, 2009; Lu et al., 2005; Wu et al., 2008b; Xiong et al., 2008; Zhang et al., 2009b). Neurorestorative agents that increase angiogenesis and neurogenesis have been shown to improve functional outcome following brain injury (Zhang et al., 2009b; Zhang and Chopp, 2009). Vascular endothelial cells within the neurovascular niche affect neurogenesis directly via contact with neural progenitor cells while soluble factors from the vascular system that are released into the CNS enhance neurogenesis via paracrine signaling (Yang et al., 2010). A better understanding of precise molecular mechanisms in the neurovascular niches will be important for developing novel angiogenic and neurogenic therapies for brain injuries.

Axonal Remodeling

The CNS has a limited capacity to regenerate after injury. Axonal sprouting from surviving neurons may be associated with spontaneous motor recovery over time after TBI (Oshima et al., 2009; Smith et al., 2007). Spontaneous pericontusional axon sprouting takes place within 1–2 weeks after TBI, which is induced by controlled cortical impact (CCI) in the adult rat but ultimately fails due to an axonal growth-inhibitory environment (Harris et al., 2010). To reduce pericontusional growth-inhibitory chondroitin sulphate proteoglycans, acute infusion of chondroitinase ABC into the site of the cortical contusion was performed, which enhanced and prolonged the sprouting response and reduced unskilled limb use deficits (Harris et al., 2010). In principle, a treatment that promotes axonal plasticity could be beneficial to functional recovery after brain injury (Smith et al., 2007). The corticospinal tract is a major fiber bundle arising from layer V pyramidal neurons of the frontal motor cortex and connects via the corticospinal or pyramidal tracts to contralateral motor neurons of the spinal cord to control voluntary movements. Collateral sprouting of the unlesioned corticospinal tract at the cervical spinal cord and neuromotor functional recovery were observed following unilateral TBI in mice (Oshima et al., 2009). In a full-thickness lesion of the forelimb region of the sensorimotor cortex, skilled paw-reaching behavior, a task that requires corticospinal function, was only partially recovered by 4 weeks. Inosine infused into the lateral ventricles for 4 weeks produced an almost complete recovery of skilled paw-reaching ability, which is associated with sprouting of the uninjured corticospinal axons across the midline into the red nucleus and cervical cord of the lesioned pathway (Smith et al., 2007).

Synaptogenesis

Brain function relies on communication among neurons through highly specialized contacts (that is, the synapses) and synaptic dysfunction plays a critical role in injury-induced defects of the CNS. In response to a CNS injury, surviving neurons reorganize their connections and form new synapses to replace those lost caused by the lesion (Becher et al., 1999). Synaptophysin (SYP), a neuronal marker of synaptogenesis, is an integral transmembrane protein component of presynaptic vesicles and is widely expressed in neurons. CCI results in the loss of specific neurons in the CA3 subfield of the ipsilateral hippocampus, resulting in partial loss of afferents to the CA1 subfield; the CNS compensates for deafferentiation by initiating synaptogenesis capable of restoring some of the lost synaptic contacts (Scheff et al., 2005). After CCI, the density of SYP signals in the injury boundary zone was less than that of the intact cortical area (Lu et al., 2004a). After treatment with atorvastatin, the density significantly increased in this area compared to the control rats (Lu et al., 2004a).

Atorvastatin may protect synapses from the impact or induce synaptogenesis in the boundary zone. Almost no SYP-positive signals were detected in the stratum lucidum and some weak signals were observed in the pyramidal cell layer. After atorvastatin treatment, intense SYP signals were found in the pyramidal cell layer as well as in the stratum lucidum (Lu et al., 2004a). Atorvastatin-induced synaptogenesis may contribute to reduction in the neurological functional deficits.

Bone Marrow Stromal Cells

Although human embryonic stem cells (hESCs) or fetal tissues are suitable sources for cell-based therapies, their clinical application is limited by both ethical considerations and other practical challenges including tumorigenicity, cell viability and antigenic compatibility. Reprogramming differentiated cells generates induced pluripotent stem cells (iPSCs) that resemble embryonic stem cells (Yamanaka, 2007). These iPSCs avoid the ethical issues and remove the major roadblock of immune rejection associated with the clinical use of hESCs, as well as potentially generate patient-specific cells for cell-replacement therapy. However, the safety and therapeutic applications of iPSCs and iPSC-derived cells must be rigorously tested in appropriate animal models before advancing to any clinical trial. The most important issue with iPSCs is potential tumorigenicity. Even with improvements in the virus-free and transgene-free reprogramming technologies, the cancer-causing possibility of the derived “safe” iPSCs/derivatives still needs to be evaluated in animal models before their clinical application for regenerative treatment.

Bone marrow stromal cells (MSCs) are a mixed cell population, including stem and progenitor cells, and are a promising source of cell-based therapy for TBI, since they can be easily isolated from many tissues and expanded in culture from patients without ethical and immune rejection problems (Chopp and Li, 2002). When grafted into the lateral ventricles of neonatal mouse brains, mouse MSCs migrated and differentiated into olfactory bulb granule cells and periventricular astrocytes (Deng et al., 2006). Systematically infused rat MSCs migrated into injured rat brains and survived (Lu et al., 2001). Some of the implanted MSCs expressed cell markers for neurons and astrocytes. Expression of the chemokine stromal-cell-derived factor-1 was significantly increased in the lesion boundary zone after brain injury induced by ischemia (Shen et al., 2007). The stromal-cell-derived factor-1 receptor, CXC-chemokine receptor-4, was expressed in MSCs both in vitro and in vivo (Shen et al., 2007). The interaction of stromal-cell-derived factor-1 with CXC-chemokine receptor-4 may contribute to the trafficking of transplanted MSCs into the injured brain (Itoh et al., 2009; Shen et al., 2007). Direct implantation (6 h post injury) of MSCs enhances neuroprotection via activation of resident NSC nuclear factor κB activity leading to an increase in interleukin-6 production and decrease in apoptosis (Walker et al., 2010). The delayed administration (24 h or 1 week following injury) of MSCs also significantly improved functional outcome in rodents following TBI (Chopp and Li, 2002; Chopp et al., 2009; Lu et al., 2001; Mahmood et al., 2004b; Mahmood et al., 2005; Mahmood et al., 2006).

MSCs secrete various growth factors, including brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF) and bFGF (basic fibroblast growth factor), and increase the levels of these factors in the brain (Chopp and Li, 2002; Mahmood et al., 2004a). MSCs also induce intrinsic parenchymal cells to produce these growth factors (Mahmood et al., 2004a). After MSC transplantation, these neurotrophic/growth factors enhance angiogenesis and vascular stabilization in the lesion boundary zone where the majority of MSCs that survive in the brain are located (Mahmood et al., 2006). These growth factors also promote neurogenesis in vitro and in vivo (Jin et al., 2002; Lee et al., 2002; Yoshimura et al., 2003). In rodent TBI models, MSCs not only increased vascular density in the lesion boundary zone and hippocampus (Qu et al., 2008), but also enhanced

neurogenesis in the SGZ and SVZ (Mahmood et al., 2004b). Delayed (4 days after TBI) treatment with MSCs alone did not reduce lesion volume, whereas MSCs seeded in collagen scaffolds significantly reduced lesion volume, enhanced the migration of MSCs into the lesion boundary zone, and significantly improved spatial learning and sensorimotor function (Lu et al., 2007a). Even more delayed (7 days post injury) transplantation of MSCs or MSCs seeded in scaffolds improved spatial learning and sensorimotor function, enhanced angiogenesis in the injured cortex and the ipsilateral hippocampus and increased transcallosal neural fibers in the injured cortex (Xiong et al., 2009). The significant therapeutic benefits of MSCs are not attributed to the few MSCs that differentiate into neural cells (Lu et al., 2001). However, MSCs appear to act as neurotrophic/growth factor generators and inducers to promote brain functional recovery via angiogenesis, neurogenesis, synaptogenesis and axonal remodeling (Chopp and Li, 2002; Chopp and Li, 2006). MSCs (or neural stem/precursor cells)-seeded scaffolds may be a new and effective strategy for treatment of TBI.

The safety and feasibility of treatment with autologous MSCs were assessed in seven patients with TBI (Zhang et al., 2008). In this trial, no toxicity related to the cell therapy was observed within the 6-month follow-up period. A safety study of autologous stem cell treatment in children with TBI has also been completed (ClinicalTrials.gov, Identifier: NCT00254722); however, no data are available. This study should determine if bone marrow harvest and re-infusion is safe in children after severe TBI.

Erythropoietin

EPO stimulates the maturation, differentiation and survival of hematopoietic progenitor cells to maintain erythropoiesis and has been widely used for treatment of anemia. Although low levels of EPO and EPO receptors exist in normal adult brains, increased expression of EPO and the EPO receptors is found in neurons, neural progenitor cells, glial cells, and endothelial cells in response to injury (Grasso et al., 2004). EPO (5000 U/kg ip) was demonstrated to cross the blood-brain barrier and to protect against brain injury in rats (Brines et al., 2000; Wang et al., 2004). Acute EPO administration (within 6-h post-TBI) provides neuroprotection (that is, decreased lesion volume and cell loss) as well as enhances neurogenesis, and subsequently improves sensorimotor and spatial learning functions in rat and mouse models (Brines et al., 2000; Cherian et al., 2007; Xiong et al., 2008; Zhang et al., 2009b). Delayed administration of EPO (5000 U/kg, ip for 14 days) from day 1 following TBI in rats significantly increased dentate gyrus neurogenesis and improved spatial memory (Lu et al., 2005). Post-TBI treatment (6-h or 24-h post-injury) with EPO (5000 U/kg) significantly increased the expression of BDNF and improved spatial learning following injury in rats (Mahmood et al., 2007b). Our recent studies demonstrate that a multiple-dose treatment with EPO (5000 U/kg/day for 3 days initiated at day 1 post-injury) is more effective than a single-dose EPO therapy in improving functional recovery in rats after TBI (Xiong et al., 2010a). Treatment with EPO also contributes to neurovascular remodeling, leading to improved neurobehavioral outcomes following TBI (Xiong et al., 2010a; Zhang et al., 2009b). EPO enhances VEGF secretion from neural progenitor cells; the treatment of such cells with EPO leads to the upregulation of VEGF receptor 2 expression in cerebral endothelial cells, promoting angiogenesis (Wang et al., 2008).

Our previous study showed that delayed (24 h post injury) EPO treatment improves neurological functional recovery without reducing lesion volume after TBI (Xiong et al., 2010a). In addition to its effects on neurogenesis and angiogenesis, EPO may improve neurological recovery partially through enhancement of axonal plasticity. Axonal sprouting from the intact corticospinal tract was increased in the denervated side of the gray matter of both cervical and lumbar levels of the spinal cord at day 35 after TBI. However, the

corticospinal tract axonal sprouting was significantly enhanced at both cervical and lumbar spinal cord in the EPO-treated TBI animals (Zhang et al., 2010). The contralesional corticospinal tract axonal sprouting was highly and positively correlated with sensorimotor recovery after TBI, suggesting axonal sprouting induced by EPO treatment may contribute to functional recovery after TBI.

In a small clinical trial for treating stroke with EPO, intravenous administration of EPO is well tolerated in acute ischemic stroke and associated with an improvement in clinical outcome at one month (Ehrenreich et al., 2002). However, in a recent large clinical trial of stroke, EPO treatment has not resulted in benefits (increasing higher mortality compared to placebo controls) (Ehrenreich et al., 2009). Combination of tissue plasminogen activator with EPO may be one of the important factors for this failed clinical trial, because a very large number of EPO-treated stroke patients also received tissue plasminogen activator treatment and combination of EPO with tissue plasminogen activator has been demonstrated to cause detrimental effects in animal models of stroke (Jia et al., 2010).

A phase III trial of EPO in patients with TBI has been planned (ClinicalTrials.gov, NCT00987454). A phase II trial investigating the safety of treatment with Darbepoetin Alfa (a long-acting form of EPO) in patients with severe TBI is ongoing (ClinicalTrials.gov, NCT00375869). A phase II/III trial to investigate the early administration of EPO to TBI patients is ongoing (ClinicalTrials.gov, NCT00260052). The high doses of EPO used for the treatment of stroke and TBI significantly increased hematocrit (Mahmood et al., 2007b; Xiong et al., 2008), which may cause adverse vascular effects such as deep venous thrombosis (Lapchak, 2008). However, non-hematopoietic EPO analogs, such as the carbamylated form of EPO (CEPO), are as effective as hematopoietic EPO in neuroprotection and are not associated with the hematopoietic side effects (Lapchak, 2008; Mahmood et al., 2007b), indicating their potential application to TBI therapy. The optimal EPO dose, dosing interval, and number of doses for reducing brain injury that promote neurorestoration and improve functional recovery have not been fully investigated after TBI. The EPO doses for the TBI clinical trials are based on stroke trials. Lack of preclinical data on these important aspects highlights the importance of fully evaluating EPO and its analogs for both acute protection and chronic restoration of function after TBI.

Statins

Statins, inhibitors of cholesterol biosynthesis used to lower cholesterol levels, induce angiogenesis, neurogenesis and synaptogenesis, and enhance functional recovery following TBI in rats (Lu et al., 2004a; Lu et al., 2004b; Lu et al., 2007b; Wu et al., 2008b). These beneficial effects of statins are independent of cholesterol-lowering action. Beneficial effects of simvastatin may be mediated through activation of Akt, Forkhead transcription factor 1 and nuclear factor–κB signaling pathways, which suppress the activation of caspase-3 and apoptotic cell death, and thereby, lead to neuronal function recovery after TBI (Wu et al., 2008a). Simvastatin activates the Akt-mediated signaling pathway, subsequently upregulating the expression of growth factors and inducing neurogenesis in the dentate gyrus of the hippocampus, thereby leading to restoration of cognitive function after TBI in rats (Wu et al., 2008b). In addition, simvastatin treatment provided long-lasting (3 month) functional improvement following TBI in rats (Mahmood et al., 2009). The protective mechanisms of statins may be partly attributed to a reduction in the inflammatory response following TBI (Li et al., 2009). When administered in combination with MSCs in a rat model of TBI, atorvastatin increased MSC access and/or survival within the injured brain and enhanced functional recovery compared with either MSC or atorvastatin monotherapy (Mahmood et al., 2007a), suggesting that statins might be used in conjunction with MSC transplantation for treating neurological disorders and injuries.

Given the wide use, favorable safety profile and positive clinical data for statins, the rare occurrence of serious adverse events and the extensive available preclinical data demonstrating neuroprotection and neurorestoration (Wible and Laskowitz, 2010), further clinical trials are warranted to determine the neuroprotective and neurorestorative properties of statins following TBI. The effect of rosuvastatin on TBI-induced cytokine change is ongoing in a phase I/II trial (ClinicalTrials.gov, NCT00990028).

Thymosin Beta 4

Thymosin beta 4 (Tβ4), a polypeptide of 43-amino acids, was first isolated from bovine thymus tissue and subsequently found to exist in all mammals studied. The major intracellular function of Tβ4 is G-actin-sequestration, which is necessary for cell motility and organogenesis (Crockford, 2007). Recent studies demonstrate that Tβ4 is a multifunctional peptide. It inhibits inflammation and apoptosis, and promotes tissue repair in skin, cornea, and heart (Morris et al., 2010b). Tβ4 is an essential paracrine factor of EPCs, and Tβ4 promotes angiogenesis after ischemic injury (Smart et al., 2007). Safety, tolerability and efficiency of Tβ4 are being evaluated in clinical patients with acute myocardial infarction (Crockford, 2007).

Tβ4 plays a critical role in many cellular processes including mobility, axonal path-finding, neurite formation, proliferation and neuronal survival (Morris et al., 2010b; Sun and Kim, 2007). Our recent study demonstrates that Tβ4 improves neurological functional recovery in mice with experimental autoimmune encephalomyelitis (Zhang et al., 2009a) and in rats with embolic stroke (Morris et al., 2010a). Tβ4 is a potential treatment for TBI. Tβ4 (6 mg/kg) was administered ip starting at day 1 and then every 3 days for an additional 4 doses to the TBI rats (Xiong et al., 2010b). Neurological functional recovery was evaluated. Animals were euthanized 35 days after injury and brain sections were stained for immunohistochemistry to assess angiogenesis, neurogenesis, and oligodendrogenesis after Tβ4 treatment. Delayed Tβ4 treatment did not affect lesion volume but significantly reduced hippocampal cell loss, enhanced angiogenesis and neurogenesis in the injured cortex and hippocampus, increased oligodendrogenesis in the CA3 region, and significantly improved sensorimotor functional recovery and spatial learning compared to the saline treatment. These data demonstrate that administration of Tβ4 significantly improves histological and functional outcomes in rats with TBI, indicating that Tβ4 has considerable therapeutic potential in TBI patients. Further investigation of Tβ4 is warranted for the treatment of TBI.

Conclusion

TBI induces angiogenesis, axonal remodeling, and neurogenesis in preclinical studies. Strategies that enhance these neurorestorative processes have been demonstrated to improve brain functional recovery in experimental TBI. A better understanding of the relationship between functional recovery and these processes will lead to novel therapeutic strategies for the treatment of TBI. The cell-based and pharmacological therapies (for example, MSCs, EPO, CEPO, statins, Tβ4, alone or in combination) described in this review induce endogenous neurorestorative processes by increasing angiogenesis, axonal remodeling, neurogenesis and synaptogenesis, and consequently improve neurological functional recovery following TBI. However, several issues should be considered during the preclinical studies and clinical trials of these strategies in TBI. Prior to the translation of an agent or cell therapy into TBI clinical trials, sufficient preclinical data should be obtained from multiple experiments, preferably in several brain injury models, on optimal administration routes, single dose versus multiple dose, bolus dose versus continuous infusion, dose-response, and therapeutic windows. Extensive pharmacokinetic data for agents to treat injured brains should also be obtained, ensuring an adequate concentration in the brain tissue. In addition,

the effective progression of strategies into clinical trials may require multiple functional agents including EPO, CEPO, statins, Tβ4, or combination therapies. These potential combinations include single agents (for example, small molecules or cytokines including EPO, CEPO, Tβ4, VEGF) with cells (for example, MSCs, NSCs, iPSCs and genetically modified derivatives) or with other approaches (biomaterial scaffolds, physical or electrical stimulation). For the safety and efficacy, the interaction of agents used in combination therapy (such as EPO combined with tissue plasminogen activator) should be fully addressed in preclinical studies before their translation to clinical trials. Although it is still important to further investigate neuroprotective treatments for TBI, an interesting novel research direction is the development of neurorestorative strategies that enhance axonal remodeling, angiogenesis, neurogenesis and synaptogenesis to improve functional recovery of the injured brain.

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