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Promoting brain remodelling and plasticity for stroke recovery: therapeutic promise and potential pitfalls of clinical translation

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Abstract

Recent laboratory findings suggest that it might be possible to promote cerebral plasticity and neurological recovery after stroke by use of exogenous pharmacological or cell-based treatments. Brain microvasculature and glial cells respond in concert to ischaemic stressors and treatment, creating an environment in which successful recovery can ensue. Neurons remote from and adjacent to the ischaemic lesion are enabled to sprout, and neural precursor cells that accumulate with cerebral microvessels in the perilesional tissue further stimulate brain plasticity and neurological recovery. These factors interact in a highly dynamic way, facilitating temporally and spatially orchestrated responses of brain networks. In view of the complexity of the systems involved, stroke treatments that stimulate and amplify these endogenous restorative mechanisms might also provoke unwanted side-effects. In experimental studies, adverse effects have been identified when neurorestorative treatments were administered to animals with severe associated illnesses, after thrombolysis with alteplase, and when therapies were initiated outside appropriate time windows. Balancing the opportunities and possible risks, we provide suggestions for the translation of restorative therapies from the laboratory to the clinic.

Introduction

Profound neurorestorative processes are induced in brain tissue in response to focal cerebral ischaemia. While neuronal plasticity is actively inhibited during adulthood, a crucial time window opens in the post-acute ischaemic phase that is characterised by intense neuronal sprouting.^{1,2} Additionally, brain capillaries sprout, and glial cells are activated to create a favourable cerebral environment for neuronal growth and plasticity.^{3,4} Thus, the injured brain exhibits a re-emergence of childhood organisational patterns, reminiscent of an ontogenetic state.⁵ The entire brain appears primed for recovery.

Clearly, this endogenous remodelling of the CNS is not sufficient to restore neurological function, and our goal is to capitalise on these recovery events to further stimulate and amplify endogenous restorative mechanisms by means of pharmacological or cell-based methods. After stroke, many patients, even elderly patients, show substantial neurological improvement.⁶ Unfortunately, the biological basis for this recovery and thus the potential for developing restorative therapies for stroke has been essentially ignored by the scientific

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Conflicts of interest

We declare that we have no conflicts of interest.

community. We have only recently gained insight into cerebral endogenous recovery processes, and thus the therapeutic potential of neurorestorative treatments was identified. Promising results from experimental studies have subsequently led to clinical trials,^{3,7–9} the results of which are eagerly awaited.

In this Review, we describe the biological substrates for neurological recovery after stroke, with a focus on neuronal plasticity, neurogenesis, and angiogenesis. The multi faceted events underlying recovery of neurological function are highly synergistic and strongly orchestrated. However, in view of the breadth of this topic and the complexity of these interactions, we will not discuss them in depth. Instead, we selectively address specific aspects of brain remodelling and plasticity, such as the role of neurogenesis and vascular changes driving neurological recovery after stroke, and omit other restorative aspects, such as the role of rehabilitation therapies, and the use of imaging, especially MRI, in monitoring cerebral structural and vascular changes indicative of recovery. We follow our description of restorative mechanisms with a discussion of translational issues, addressing the question of how recent laboratory findings of the therapeutic stimulation of brain plasticity might be moved to the clinic, and describing potential pitfalls of clinical translation.

Benefits of neurorestorative therapies

By contrast with neuroprotection, which requires an acute intervention, treatments promoting neurological recovery by remodelling of brain tissue can be instituted during the stroke recovery phase.^{10–15} Thus, a comparably extended time window exists in which brain remodelling could be therapeutically modulated. In rodents, beneficial effects were reported even when treatments were initiated days, weeks, or months after stroke.^{14–20} By sharp contrast with neuroprotection, the efficacy of neuro restorative therapies does not depend on the successful reperfusion of ischaemic brain tissue.^{11,12,15,17,21} Neuroprotective agents must be delivered to the site of ischaemic injury, which by definition includes tissue that is highly underperfused. Neurorestorative therapies target viable tissue with adequate and intact perfusion. In view of the long therapeutic time window and the fact that tissue reperfusion is not a requirement, neuro restorative therapies can potentially benefit all stroke patients.

Biological substrates for neurological recovery

Many endogenous restorative responses, including neuronal plasticity, neurogenesis, and angiogenesis, can be targeted with pharmacological or cell-based treatments to stimulate neurological recovery after stroke.

Neuronal plasticity

Stroke induces anterograde Wallerian degeneration of pyramidal tract axons, the degree of which depends on the severity of the ischaemic insult (figure 1).^{19,21,22} Subsequently, axonal fibre tracts reorganise along the infarct rim.^{21,23} Surviving pyramidal tract axons distal to the site of ischaemic injury sprout.¹⁹ Terminal axonal sprouting is enhanced in both the ipsilesional^{19,24} and the contra lesional^{12,19} pyramidal tract system. In addition to the sprouting of pyramidal tract fibres, remodelling of transcallosal projections connecting the two motor cortices occurs.^{14,25} Post-ischaemic endogenous plasticity partly compensates for the loss of axons in target structures of damaged fibre bundles at various levels of the brain (eg, red nucleus and facial nucleus) and spinal cord.^{12,19,24}

In rodents, the survival of ischaemic pyramidal tract axons can be improved by pharmacological and cell-based treatments.^{26–28} Importantly, neurorestorative treatments do not necessarily promote the growth of terminal axons distal to the site of injury, as was

shown after delivery of the growth factors erythropoietin and vascular endothelial growth factor (VEGF).^{19,24} Instead, homologous fibres originating from the contralesional motor cortex grow out across the midline to reach denervated neurons in target structures of the lesioned pyramidal tract (figure 1).^{10–12,15,17,19,24} This lesion-remote plasticity involves the midline crossing of fibres from the contralesional to the ipsilesional hemisphere at the level of the red nucleus^{10,17,19,24} and the re-entry of fibres into the contralesional hemicord at the level of the cervical spinal cord (figure 1).^{11,12,15}

Importantly, axonal growth responses are relatively uniform for different types of treatment: contralesional sprouting was evident after delivery of growth factors,^{19,24} neutralising antibodies directed against the axonal growth inhibitor NogoA,^{11,17} neurostimulants (eg, amphetamine),²⁹ and cell-based therapeutics, namely bone-marrow-derived stromal cells^{12,30} or neural precursor cells.¹⁵ Contralesional sprouting after treatment with growth factors is associated with profound alterations of gene expression, including the downregulation of axonal growth inhibitors^{19,24} and inflammatory signals^{19,31} in the vicinity of the contralesional pyramidal tract.

Contralesional plasticity as a basis for neurological recovery occurs in several species (eg, in rats,^{11,15,17} mice,^{12,19,24} and macaques³²). Enhanced plasticity and neurological recovery after stroke were evident not only in young but also in older animals.^{17,26,33} After treatment with bone-marrow-derived stromal cells, structural effects on the preservation of corticospinal axons persisted for up to 1 year.²⁶ Thus, evidence for axonal remodelling in response to stroke that is amplified by restorative therapy is robust in the laboratory setting.

Neurogenesis

Neurogenesis is stimulated in response to stroke and is substantially amplified by therapeutic interventions that promote functional recovery.^{33–36} Neurogenesis is sustained in the germinal niches in the subventricular zone of the lateral ventricle and the subgranular layer of the dentate gyrus, which host neural precursor cells even during adulthood.^{34,35} When a stroke occurs, these cells proliferate and migrate in the direction of the ischaemic lesion, propagating in chains along blood vessels.^{37,38} The question thus arises as to whether neurogenesis per se promotes brain recovery and, if so, whether its restorative effect can be attributed to cell replacement.

The survival rate of adult neural precursor cells in the brain parenchyma is poor and only a small percentage of the cells differentiate into neurons.^{35,39–42} This fact raises doubts about the contribution of neuronal cell replacement to neurological recovery. Meanwhile, evidence that neurogenesis contributes to functional neurological recovery has been obtained in studies with transgenic ablation of neural precursor cells expressing the marker doublecortin⁴³ and in studies with exogenous delivery of adult neural precursor cells,^{14,15,44} which reported impaired or enhanced neurological recovery, respectively. Unfortunately, the studies of neural precursor cell ablation focused on structural tissue preservation in the first 24 h after stroke.⁴³ Additional experiments are needed to define the role of neurogenesis in the post-acute stroke phase.

The finding that neural precursor cells prevent ischaemic injury and promote neurological recovery could be attributed to bystander effects related to the interaction of neuroblasts with the microvasculature in the vicinity of the ischaemic lesion, which creates an environment that promotes brain remodelling (figure 2).^{14,15,37,45} Microvessels and neuroblasts mutually support each other via release of trophic factors, resulting in a tight interplay that stimulates brain remodelling processes, reducing delayed neuronal degeneration,^{14,45} promoting neuronal plasticity,¹⁵ modulating glial responses,^{14,46} and attenuating brain inflammation^{14,46} (figure 2).

Angiogenesis

After stroke, angiogenic factors are expressed by neurons and astrocytes, which induce microvascular growth.^{4,47–50} Acting through VEGF receptor 2 (VEGFR2), which is localised at high concentrations at the tip of newly formed capillaries, VEGF induces endothelial proliferation,^{4,47–49} and concurrently upregulates the transmembrane ligand delta-like ligand 4, which signals to microvascular cells in the capillary stalk, to downregulate VEGFR2 via interaction with its receptor Notch 1, thus preventing uncontrolled sprouting of brain capillaries.^{51,52} This lateral inhibition, which is a key process underlying structural development during ontogeny, is recapitulated after ischaemia.^{53–55}

In patients, a close relation exists between blood flow and neurological improvement after stroke.⁵⁶ This enhanced perfusion directly provides for the metabolic needs of the tissue.⁴ Newly formed microvessels exert trophic influences on the brain parenchyma via release of growth factors, such as BDNF.⁵⁷ In the peri-infarct tissue, newly formed vessels create a milieu for the migration, homing, and neuronal differentiation of neuroblasts via release of stromal derived factor 1, VEGF, and matrix metalloproteinases 2 and 9, which attract neuroblasts.^{58,59} Importantly, neuroblasts also reinforce angiogenesis via release of VEGF.⁴⁵ As such, microvascular sprouting and neurogenesis bidirectionally promote each other (figure 2). The interactions between endothelial cells and neuroblasts are especially strong during the first month after stroke.^{57,58}

The serine protease activated protein C enhances this neurovascular interplay, stimulating endothelial proliferation and tube formation⁶⁰ and inducing proliferation and migration of neural precursor cells⁶¹ via protease-activated receptor 1. Studies on angiogenesis suggest that new vessel formation has roles in ischaemic brain remodelling after stroke that are independent of blood flow, and that angiogenesis contributes to stroke recovery in a substantive way.

Glial cells in brain remodelling and plasticity

Glial cells, especially astrocytes, control brain homeostasis and create a microenvironment for successful brain remodelling. Astrocytes also remove excitatory neurotransmitters (eg, glutamate) and electrolytes (eg, potassium) from the extracellular space, thus controlling neuronal excitability and enabling synaptic plasticity.⁶² In addition to these cell-specific actions, astrocytes and oligodendrocytes release proteins and proteoglycans into the extracellular space that directly modulate neuronal plasticity and growth.

In healthy tissue, chondroitin sulphate proteoglycans are secreted by both astrocytes and oligodendrocytes to create a growth-repulsive microenvironment for axons and dendrites.^{1,63} In response to focal cerebral ischaemia, these proteoglycans are downregulated in areas exhibiting axonal growth over a period of 2–3 weeks after stroke.⁶⁴ Therapeutic interventions promoting axonal plasticity, such as delivery of VEGF or bone-marrow-derived stromal cells, further decrease proteoglycan levels.^{24,65}

Subsequent to focal cerebral ischaemia, and in parallel to the downregulation of growth-repulsive proteoglycans, astrocytes de novo express neurotrophic factors, such as glial-cell-line-derived neurotrophic factor (GDNF)⁶⁶ and VEGF,⁶⁷ and serine proteases, namely tissue plasminogen activator (tPA).⁶⁸ Delivery of bone-marrow-derived stromal cells or mesenchymal stromal cells was shown to further increase levels of GDNF⁶⁶ and tPA^{68,69} and reduce levels of the tPA inhibitor plasminogen activator inhibitor 1.⁷⁰ Astrocytes and oligodendrocytes provide basic trophic support that is independent of brain region or surrounding neuronal populations, thus setting the stage for brain plasticity processes (figure 3).

Signals controlling axonal and dendritic sprouting

The dynamic responses of neurons after stroke require efficient mechanisms to prevent uncontrolled axonal or dendritic growth, thus ensuring that maladaptive sprouting does not occur. By contrast with the astrocytic and oligodendroglial proteoglycans, neuronal growth signals have a high degree of specificity for some but not other neuronal populations and for defined plasticity processes (figure 3). The tumour suppressor phosphatase and tensin homolog (PTEN) and its downstream target mammalian target of rapamycin (mTOR), for example, were shown to induce the sprouting of apical but not basal dendrites of mouse cortical layer 2/3 pyramidal neurons.⁷¹ PTEN and mTOR were not involved in induction of dendritic plasticity in layer 5 pyramidal neurons.⁷¹

Neuronal growth responses include sets of signals that have to be activated synergistically so that axonal outgrowth can occur. In the rat somatosensory whisker barrel field, the ATP-dependent DNA-modifying enzyme α -thalassaemia/mental retardation syndrome X-linked (ATRX) protein, which controls neuronal survival and migration during brain development, contributes to the formation of axonal growth cones, which are stabilised by insulin-like growth factor 1.⁷² The spatial orientation and distribution of fibres were shown to be controlled by leucine-rich repeat and IgG domain containing protein 1 (Lingo1) and Nogo receptor 1, which directed the newly formed axons to cortical areas that were otherwise not targeted.⁷²

Axonal sprouting of cortical neurons contralateral to the stroke has specific electrophysiological accompaniments characterised by low-frequency synchronised neuronal activity in the range of 0.2–2.0 Hz on day 1 and of 0.1–0.4 Hz on days 2–3.⁷³ In the peri-infarct cortex of the lesioned hemisphere, tonic inhibition of layer 2/3 pyramidal neurons was present from 3–14 days after stroke and was mediated by extrasynaptic GABA_A receptors.⁷⁴ A pharmacological antagonist of the GABA_A receptor $\alpha 5$ subunit prevented the excessive inhibition, as did genetic deletion of the GABA_A receptor $\alpha 5$ or δ subunits, and improved stroke recovery.⁷⁴

Glutamatergic AMPA receptors contribute to learning-induced motor plasticity. Subsequent to ischaemic stroke, AMPA receptor activation by pharmacological allosteric modulators evokes the release of BDNF in the peri-infarct cortex, thus enhancing motor recovery.⁷⁵ AMPA receptor antagonists impair neurological outcome, as does blockade of BDNF by TrkB-Fc-impregnated hydrogel.⁷⁵ Neuronal activity induces temporally and spatially tuned responses in the brain (figure 3). The continuous rehearsal of activation patterns provides the basis for temporally and spatially organised axonal growth (figure 3), an understanding of which could allow the development of rehabilitation strategies to reinforce the effects of neurorestorative therapies.

Potential limitations of neurorestorative therapies

The promotion of brain remodelling and plasticity represents a paradigm shift from the concept of simple structural tissue preservation. From a systems biological perspective, an intervention that modulates endogenous restorative responses, such as axonal growth, neurogenesis, and angiogenesis, will probably alter tissue homeostasis and could lead to adverse side-effects. In view of the complexity of the systems involved, we need to take steps to make sure that adverse side-effects do not outweigh the benefits of treatment.

Compromised brain remodelling and plasticity associated with age

Stroke affects human beings mainly in the later stages of life. Aged rats respond to plasticity-promoting therapies, but age might have an effect on some of the processes targeted by neurorestorative interventions. Improved neurological recovery associated with

preservation of pyramidal tract axons ipsilateral to the stroke and enhanced pyramidal tract sprouting contralateral to the stroke was identified in 25-month-old or 12-month-old rats with ischaemia treated with neutralising anti-NogoA antibodies,¹⁷ pharmacological compounds,⁷⁶ or bone-marrow-derived stromal cells.²⁶ In the case of treatment with bone-marrow-derived stromal cells, neurological improvements persisted in middle-aged rats (12 months of age) for at least 1 year.²⁶ Although neurological recovery was successful, dendritic and synaptic plasticity of hippocampal CA3 and CA1 pyramidal and dentate gyrus granule cells was not influenced by anti-NogoA antibodies in old rats (24 months of age), yet improvements in spatial memory were reported.⁷⁷ Indeed, the expression of plasticity-related proteins in neurons differs between young and old animals. Thus, insulin-like growth factor 1 was localised mainly to astrocytes in the periinfarct cortex of 2-month-old rats, but exhibited predominantly neuronal labelling in 2-year-old rats.⁷² Lingo1 immuno staining, by contrast, was stronger in neurons of old rats (2 years of age) than in young rats (2 months of age).⁷²

An effect of age was not only seen for neuronal sprouting, but also for neurogenesis and angiogenesis. The numbers of proliferating neural precursor cells in the subventricular zone and subgranular layer were lower in the brain tissue of 15-month-old rats than in that of 3-month-old rats, both under normal physiological and ischaemic conditions.⁷⁸ Although the *de novo* generation of neurons in the ischaemic striatum was very similar in both groups, neurogenesis was decreased in the dentate gyrus of 15-month-old rats when exposed to focal cerebral ischemia.⁷⁸ Reduced neurogenesis in old animals could be related to lower expression of VEGFR2 on the surface of neural precursor cells, as reported in 2-year-old mice compared with 3-month-old or 12-month-old mice.⁷⁹ After delivery of an adeno-associated viral VEGF vector, both proliferation of neural precursor cells and formation of brain capillaries were compromised in old mice (24 months).⁷⁹ Although evidence is limited to a rather small number of studies, the preserved neurological recovery in old animals argues against specific age limits for neurorestorative treatments. Despite this evidence, the effects of age need to be controlled in clinical proof-of-concept studies.

Compromised brain remodelling and plasticity associated with vascular risk factors

In addition to ischaemic stroke, ageing is associated with other systemic and vascular diseases. Experimental studies poorly mimic comorbidities, because experiments are done mainly in animals that are otherwise healthy. The relevance of associated diseases for the efficacy of plasticity-promoting therapies was recently shown in rats with streptozotocin-induced diabetes.⁸⁰ Paradoxically, treatment with bone-marrow-derived stromal cells did not improve neurological recovery in rats with diabetes, but increased mortality, blood-brain barrier leakage, and brain haemorrhage.⁸⁰ In histochemical studies, neointima formation and arteriole narrowing were exacerbated by bone-marrow-derived stromal cells in rats with diabetes, as was macrophage accumulation in blood vessels.⁸⁰ These abnormalities were attributed to increased angiogenin expression in the brain and brain-supplying arteries of rats with diabetes.⁸⁰ Investigators suggest that treatment with bone-marrow-derived stromal cells should not be considered in patients with diabetes.

Diabetes has especially detrimental effects on the vascular system, but with a prevalence of one in four patients it is not the most prevalent risk factor in ischaemic stroke populations.⁸¹ Three-quarters of stroke patients have arterial hypertension, and about half of patients have hypercholesterolaemia.⁸¹ The consequences of both risk factors for brain remodelling are not completely understood. In spontaneously hypertensive rats, subtle abnormalities in the expression of neurotrophic factors and their receptors, namely reduced levels of BDNF, neutrophins 3 and 4, TrkA, and TrkB, have been described in the dentate gyrus.⁸² In focal cerebral ischaemia, spontaneously hypertensive rats had preserved contralesional pyramidal tract sprouting in response to treatment with neutralising antibodies directed against NogoA,

which was consistent with functional neurological improvements that were very similar to those in non-hypertensive rats.¹¹ This preserved response to NogoA antibodies suggests that the growth factor abnormalities might not be clinically relevant. Whether this conclusion is true for prolonged arterial hypertension, which causes cerebral microangiopathy in human beings, remains to be shown.

Hypercholesterolaemia reduces angiogenesis⁸³ and promotes blood–brain barrier permeability.⁸⁴ These vascular changes are driven by many factors, including reduced endothelial NO synthase (eNOS) activity, excessive lipid peroxidation, and overactivation of matrix metalloproteinases 2 and 9, calpain 1 and 2, and the small RhoGTPase RhoA.^{83,84} In rats with cerebral ischaemia, vitamin B3 administration, which elevates high-density lipoprotein and thereby reduces serum cholesterol, increased angiogenesis, the expression of VEGF and angiopoietin 1, and enhanced the phosphorylation (ie, activation) of eNOS and the angiopoietin-1 receptor Tie2, thus improving neurological recovery.⁸⁵ In cerebral endothelial cell culture, the effects of vitamin B3 on capillary tube formation were reduced after eNOS inhibition or Tie2 knockdown,⁸⁵ showing a role for eNOS and angiopoietin 1 in the beneficial effects of the high-density lipoprotein elevation.

Although evidence is limited, recent studies suggest that impaired angiogenesis in patients with hypercholesterolaemia parallels disturbances in synaptic plasticity. In hypercholesterolaemic mice lacking scavenger receptor class B type I, age-related deficits of spatial memory have been described that were accompanied by impaired long-term potentiation of hippocampal CA1 neurons.⁸⁶ In mice receiving a high-fat diet, contextual fear conditioning and passive avoidance deficits were associated with reduced CA1 long-term potentiation and long-term depression.⁸⁷ After focal cerebral ischaemia in rats, vitamin B3 was used to increase high-density lipoprotein concentrations, which enhanced white matter remodelling in the peri-infarct tissue, increased BDNF and TrkB levels, and down regulated Nogo-receptor levels.⁸⁸ In view of the high prevalence of hypercholesterolaemia in stroke patients, blood lipids might be a suitable target for neurorestorative therapies. Lipid-lowering drugs, especially statins, are widely prescribed for stroke patients as secondary stroke prevention. Statins also have neurorestorative properties (ie, promotion of neuronal survival, angiogenesis, and neurogenesis).^{36,89} Whether the modulation of blood lipids can be used in the clinical setting to promote the recovery of stroke patients with and without hyper cholesterolaemia remains to be shown.

Drug interactions with the thrombolytic drug alteplase

The thrombolytic drug alteplase might compromise restorative stroke treatments, as shown in the recent German multicentre erythropoietin trial,⁹⁰ in which 522 patients were randomly assigned to receive placebo or erythropoietin within 6 h after ischaemic stroke. 331 (63%) patients in this study received alteplase treatment. Unexpectedly, erythropoietin did not improve clinical outcome in stroke patients but increased the risk of serious complications, namely death, bleeding, oedema, and thromboembolic events in patients who received alteplase.⁹⁰ Subsequent experimental studies have shown that erythropoietin activated matrix metalloproteinases 2 and 9 after focal cerebral ischaemia, an effect that was potentiated by alteplase, thus provoking blood–brain barrier leakage, brain oedema, and haemorrhagic transformation of brain infarcts.^{91,92} Neurorestorative treatments should be assessed very carefully in patients treated with alteplase. In these patients, proof-of-concept studies should only be initiated in the acute-stroke phase once animal data support the safety of this combination treatment.

Timing of treatment and crosslinks between neuronal plasticity and death

The promotion of brain remodelling and plasticity is not limited to a sharp and well defined therapeutic window.^{1–3} Yet, because of the neurovascular remodelling processes activated in response to stroke, a time interval of a few days to months after stroke exists in which neurological recovery presumably is most promising. The possibility of unfavourable effects of specific neurorestorative treatments when given at an inappropriate timepoint was suggested in a recent study of the effects of NogoA deactivation after transient focal cerebral ischaemia in mice.⁹³ NogoA deactivation initiated by genetic knockout or by intracerebral ventricular delivery of neutralising antibodies decreased neuronal survival and delayed neuronal recovery when the deactivation was constitutive or initiated 24 h before the stroke.⁹³ Importantly, no effect on neuronal survival was reported when anti-NogoA antibodies were delivered after focal cerebral ischaemia.⁹³ Similar findings about the importance of timing for administration of a neurorestorative therapy for stroke have been reported for VEGF⁹⁴ and modulators of matrix metallo proteinases.⁹⁵

Crosslinks between neuronal plasticity and death have also been described for CD95 (Apo1/Fas). In neurons and neural precursor cells, CD95 is not a death receptor.⁹⁶ Instead, CD95 activation was shown to promote survival and differentiation of neural precursor cells, resulting in enhanced recovery of working memory in mice with global cerebral ischaemia.⁹⁷ In embryonic hippocampal and cortical neurons, activation of CD95 stimulated neurite branching, which occurred in a caspase-independent and death-domain-dependent manner and was paralleled by an increase in non-phosphorylated Tau.⁹⁸ Enhanced neurogenesis is associated with enhanced neuronal survival in most but not all conditions: in a recent study examining effects of ephrin-B3 deficiency, enhanced proliferation and survival of endogenous neural precursor cells but exacerbation of ischaemic injury were reported.⁹⁹ Neural surface receptors integrate signals with diverse functions, such as neuronal injury and repair,⁹⁶ which depending on the pathophysiological context could have Janus-like roles in the ischaemic brain.^{5,100} Proof-of-concept studies should thoroughly examine effects of neurorestorative therapies on structural tissue integrity, especially when treatments are initiated early after stroke.

Consequences for clinical translation

In view of several discoveries showing the potential of neurons, brain microvascular cells, and glial cells to respond to pharmacological or cell-based restorative treatments under conditions of focal cerebral ischaemia, we are poised to promote stroke recovery in human beings. Neurons, cerebral microvascular cells, and glial cells respond synergistically to therapeutic interventions, creating an environment in which successful recovery can take place. Our current knowledge derives largely from laboratory animals, mainly rodents. With respect to clinical translation, these studies have two important limitations: the predominance of studies in young, otherwise healthy animals and the scarcity of studies in non-human primates. Although restorative treatments can promote neurological recovery in old animals,^{17,26,72,76–79} specific findings in ageing animals^{72,77–79} and in animals with vascular risk factors^{80,82,86,87} suggest that the efficacy of new therapeutics might be compromised in elderly patients with risk factors.

The need for primate studies relates to the organisation of neuronal networks, which is far more complex in human beings than in rodents. It is not clear how the more complex brain structure, which is reflected by a larger number of network interactions (as a consequence of a higher number of neurons) and a stronger role of cortical systems in the control of CNS function (panel 1), affects the remodelling of brain tissue in human beings. Fibre bundles are frequently affected by strokes, in human beings, because of the large contribution of white matter to the whole brain volume (panel 1) and the common manifestation of strokes in deep

brain structures. An important species difference relates to the anatomical size of the brain, which is about an order of magnitude larger (in terms of length measures) in human beings than in rodents (panel 1). Since sprouting axons need to cover larger distances in human beings than in rodents, the contribution of axonal plasticity to neurological recovery might be diminished. Solid evidence exists for a contribution of pyramidal tract remodelling to functional recovery in stroke patients.^{101,102} However, this evidence results from indirect outcome measures, such as MRI¹⁰¹ and electroencephalography,¹⁰² which provide information about the macroscopic (ie, tissue), but not microscopic (ie, cellular or even subcellular) level.

Although neurorestoration after stroke in animals seems relevant to human beings, testing restorative therapy in people is far more complex than in laboratory animals. Although experimental studies make use of defined pathophysiological states, to assess comparably uniform stroke lesions with defined topography in homogeneous, often genetically inbred, animals, the clinical setting is much more diverse and complex than are laboratory conditions. Stroke patients vary substantially in age and genetic background, as well as in the aetiology, localisation, and size of brain infarcts (panel 1). Genetic influences on corticospinal plasticity have recently been shown in human beings by using transcranial magnetic stimulation techniques, whereby healthy patients carrying the Val66Met polymorphism in the *BDNF* gene exhibited significantly smaller training-induced increases in the amplitude of motor evoked potentials.¹⁰³ Thus, the question arises as to whether clinical neurorestoration studies should enrol all stroke patients independent of genetic backgrounds, stroke localisation, and stroke size, or whether studies should focus on patients in more defined subgroups. Heterogeneous patient populations could be a reason for the failure of therapeutic interventions because they might dilute the benefits of treatment.

Animal studies have identified medical conditions that specifically undermine neurorestorative therapies by making the brain prone to detrimental responses. Such findings have been reported for severe associated systemic diseases (ie, diabetic vasculopathy),⁸⁰ subsequent to systemic thrombolysis,^{91,92} and when restorative treatments were initiated at inappropriate timepoints (ie, before stroke;⁹³ table). In each of these cases, the therapeutic intervention was associated with a deterioration of neurological state and structural brain damage (panel 2). In view of the Janus-like role of brain-repair signals for neuronal injury,^{5,100} the initiation of neurorestorative therapies in the very acute phase of stroke would probably induce more undesirable effects than would treatment in the very late (chronic) phase. Despite the robust evidence for specific time windows in which neuronal plasticity and angiogenesis are most active,^{64,66,67} neurorestorative treatments were still successful in animals once brain remodelling was induced months after the stroke.^{18,20} This finding paves the way for neurorestorative therapies far into the rehabilitation phase.

Conclusions

What should the next step be in the translation of restorative stroke therapies from the laboratory to the clinic? The benefits of restorative therapy are evident. With these therapies, we can essentially treat all patients, and we are not hampered by reduced tissue perfusion, which restricts delivery of neuroprotective agents, nor by the requirement of rapid intervention, within hours after stroke. Yet, restorative therapy, although a clinical imperative, requires stringent proof-of-concept studies, translating in a one-to-one manner experimental findings to the patient. If not properly translated, this promising approach, for which compelling experimental data have been accrued, could become interred with the myriad of failed neuroprotective trials. Confounding influences related to the age and genetics of stroke patients and stroke localisation and size, as well as adverse effects driven by comorbidities, concomitant acute treatments (such as thrombolysis), and treatment

timing, must be considered and carefully assessed. A robust biological substrate to drive functional improvements is present in many stroke patients, even in elderly people. Our ability to amplify these endogenous processes by means of restorative therapies, whether cell-based or pharmacological, we believe, will have a profound effect on stroke treatment in the future.

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Panel 1: Factors impeding clinical translation of neurorestorative therapies

Patients have many characteristics that differ from laboratory animals and impede the translation of therapies from bench to bedside. Characteristics of human beings that differ from rodents—the most widely used laboratory animals—include:

Complexity of brain

- Large number of network interactions
- Strong contribution of white matter to brain volume
- Eminent role of cortical systems in control of brain function

Size of brain

- Long fibre tracts (eg, corticospinal tract)

Heterogeneity of patients

- Heterogeneous age cohorts
- Heterogeneous patient genetics

Heterogeneity of strokes

- Heterogeneous stroke localisation (eg, cortical or subcortical, predominantly affecting neurons or white matter fibres, respectively)
- Heterogeneous stroke size

Heterogeneity of associated risk factors and disorders

- Associated vascular risk factors and illnesses (eg, diabetes)

Panel 2: Possible causes of detrimental effects associated with neurorestorative therapy

On the basis of animal studies, detrimental effects of neurorestorative therapies could have three different causes, which should carefully be ruled out in proof-of-concept studies:

1. The treatment triggers a response that would be beneficial for an otherwise healthy organism. However, because of concomitant diseases, the therapeutic stimulus exacerbates brain injury and is detrimental to recovery (eg, detrimental effects of bone-marrow-derived stromal cells in rats with diabetes).⁸⁰
2. The treatment triggers its intended response, but its actions compete with a response to another treatment given for different purposes, resulting in excessive responses of the brain tissue that compromise tissue viability (eg, exacerbation of blood–brain barrier breakdown by erythropoietin when given after alteplase-induced thrombolysis).^{91,92}
3. The treatment triggers a response that is ambiguous because treatment is initiated in a context that fails to meet requirements for neurorestorative therapies. The organism responds to the stimulus in an unexpected way, owing to its inability to interpret the stimulus correctly (eg, induction of neuronal death when brain remodelling is induced before ischaemia).⁹³

In all three settings, adverse effects of a given therapy could outweigh the beneficial effects, resulting in a lack of clinical efficacy.

Search strategy and selection criteria

We searched PubMed for reports about ischaemic stroke published between January, 1975, and February 2012, with the search terms “plasticity”, “remodeling”, “restorative”, “angiogenesis”, “neurogenesis”, and “axonal growth”. Only papers published in English were reviewed.

Figure 1. Contribution of lesion-remote plasticity to neurorestorative actions of pharmacological and cell-based therapies

(A) After ischaemia, injured axons that are part of the ipsilesional pyramidal tract degenerate. Brain tissue surrounding the infarct rim reorganises, and transcallosal fibres originating from the ipsilesional and contralesional motor cortex sprout. After treatment, the responses of transcallosal fibres are amplified. Fibres originating from the contralesional motor cortex grow out across the midline to innervate denervated target structures in the midbrain, brainstem, and spinal cord. (B) Example of corticobulbar plasticity at the level of the facial nucleus induced by the growth factor erythropoietin, which was intracerebroventricularly delivered in the subacute stroke phase, starting 3 days after

ischaemia. Note the increase in fibres branching off the contralesional pyramidal tract to innervate the contralesional facial nucleus. Axonal fibre densities were assessed 7 weeks after the initiation of treatment by the anterograde tract tracer biotinylated dextran amine, which was injected into the contralesional motor cortex. (C) Representative microphotographs of fibres emanating from the pyramidal tract. Adapted from Reitmeir and colleagues,¹⁹ by permission of Oxford University Press. Data are mean (SD). * $p < 0.05$ compared with non-ischaemic vehicle. † $p < 0.05$ with ischaemic vehicle, ANOVA followed by least significant difference tests.

Figure 2. Interaction of microvascular cells with neuroblasts during post-ischaemic brain remodelling

Following ischaemia, neuroblasts (green [DCX-GFP] in upper micrograph) closely associate with cerebral microvessels (lumen stained in red [rhodamine-labelled dextran, systemically injected before animal sacrifice]; nuclei counterstained in blue [DAPI]). Via release of growth and differentiation factors, microvascular cells and neuroblasts foster neuronal survival and plasticity, and modulate glial responses and immune reactions in the brain. CD45=CD45 antigen. DAPI=4',6-diamidino-2-phenylindole. DCX-GFP=green-fluorescent-protein-labelled doublecortin. GFAP=glial acidic fibrillary protein. Tuj-1=neuron-specific class III β -tubulin.

Figure 3. Effect of neuronal activity on brain environment, neuronal signalling, and axonal growth

Glial cells, blood vessels, and neuroblasts provide basic trophic support, having rather low selectivity for brain regions and neuronal populations. Together, they represent the stage for plasticity processes in the brain. Neuronal activity, which is temporospatially tuned and specific to the brain region and cell type, reshapes this environment, enabling temporally and spatially organised axonal growth, and promoting the proficiency and virtuosity of brain networks.

Table

Examples of undesirable actions of neurorestorative therapies based on findings in animal studies

	Treatment conditions	Outcome in animal studies
Severe associated diseases	Bone-marrow-derived stromal cells in diabetic vasculopathy	Increased mortality, blood–brain barrier leakage, brain haemorrhage ⁸⁰
Treatment subsequent to systemic thrombolysis	Combination therapy with erythropoietin and alteplase	Increased blood–brain barrier leakage, brain oedema, brain haemorrhage ^{91,92}
Treatment initiated at wrong timepoint	Delivery of neutralising anti-NogoA antibodies before transient focal cerebral ischaemia	Exacerbation of neuronal injury ⁹³