How Can You Mend a Broken Brain? – Neurorestorative Approaches to Stroke Recovery

Dale Corbett^{a–d} Carine Nguemeni^{a, b} Mariana Gomez-Smith^{a, b}^aDepartment of Cellular & Molecular Medicine, Faculty of Medicine, ^bCanadian Partnership for Stroke Recovery, University of Ottawa, Ottawa, Ont., ^cFaculty of Medicine, Memorial University of Newfoundland, St. John's, Nfld., and ^dFaculty of Medicine, University of Toronto, Toronto, Ont., Canada

Key Words

Stroke recovery · Neuroplasticity · Enriched rehabilitation · Functional outcome

Abstract

Background: Stroke is a devastating disorder that strikes approximately 15 million people worldwide. While most patients survive stroke, many are left with lifelong impairments, thereby making stroke the leading cause of permanent neurological disability. Despite this, there are a few options for treatment of acute stroke. Restoration of blood flow using clot-dissolving drugs has produced impressive benefits in some patients. However, for these drugs to be effective, they must be given soon after stroke onset and relatively only a few stroke patients reach hospital within this time. Side effects of these compounds further limit their use. **Summary:** Enhancing the brain's endogenous capacity for reorganization and self-repair offers the most promise for victims of stroke. Indeed, many stroke patients show considerable spontaneous functional improvement. Findings in the last 15 years suggest that stroke and related injury create a cerebral milieu similar to that of early brain development, a period characterized by rapid neuronal growth and neuroplasticity. A variety of interventions (e.g., stem cells, delivery of growth factors) are currently being explored in order to en-

hance neuroplasticity and reorganizational processes that are important for recovery of function. An emerging concept is that combinational or 'cocktail' therapies are more effective than single interventions in improving stroke recovery. Among these, one of the most promising therapies is enriched rehabilitation, a combination of environmental enrichment and task-specific therapy (e.g., reach training). **Key Messages:** Neurorestorative approaches to brain reorganization and repair are providing new insights into how neural circuits respond to injury and how this knowledge can be used for optimizing stroke rehabilitation practice.

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Introduction

Stroke is the leading cause of adult neurological disability and the second leading cause of death worldwide. Attempts to reduce the incidence of stroke are being countered by an aging population and rising rates of obesity and metabolic disturbances as a result of a sedentary lifestyle and poor diet [1]. Despite significant advances in our understanding of the pathophysiology of stroke, all neuroprotective drug trials failed leaving t-PA as the only effective treatment for acute stroke [2]. Unfortunately, the limited time window and side effects limit its use to a

relatively small percentage of stroke patients [3]. Furthermore, many patients benefitting from t-PA are still left with significant disabilities. In recent years, research in the stroke field has begun to shift from acute stroke to the stroke recovery phase. This change in emphasis has been triggered by a dramatic increase in our understanding of neuroplasticity and its potential to reorganize and restore function to the stroke-damaged brain [4–8, 26]. Clearly, neurorestorative interventions, including rehabilitation, offer the most hope for stroke survivors because the therapeutic time window is not limited to minutes to hours, as for t-PA, but instead applicable to weeks, months, and years and can be applied to any type of stroke, including hemorrhagic stroke.

Modeling Stroke Recovery

An important lesson from the failed stroke neuroprotection trials is that preclinical models, while never perfectly mimicking human stroke, must nonetheless capture essential features of the disease in order to avoid generating false positive results with subsequent translational failure [9–11]. For example, most stroke patients have residual deficits even years after their injury [12]. Thus, it is essential to develop animal models that also produce similar chronic functional deficits. An increasingly popular model uses endothelin-1 (ET-1), which when stereotactically injected induces vasoconstriction followed by a gradual reperfusion, similar to human ischemic stroke [13]. The ensuing long-lasting, sensory-motor deficits make it ideal for stroke recovery studies. In addition to choosing an appropriate stroke model, it is essential to employ a sensitive battery of sensory-motor tests since recovery on one test does not guarantee recovery on other tests. Impairment of the upper limb after stroke is very common and the deficits are often persistent [14]. Therefore, a reaching task should be part of any rodent behavioral test battery to assess stroke recovery. Particularly useful, due to their great sensitivity and clinical relevance, are tests of skilled reaching such as the Montoya staircase or single pellet reaching test [4, 14–17].

Stroke Rehabilitation – Missed Opportunities?

An emerging view of the last 15 years is that stroke re-creates a cerebral milieu similar to that of early brain development, a period characterized by rapid brain growth and remodeling of synaptic connections. The adult brain re-

sponds to stroke by increasing dendritic growth, forming new synapses, reorganizing functional maps, and by up-regulating growth factors [6, 18–20] – all processes thought to contribute to behavioral recovery. Complex changes in growth-promoting and growth-inhibitory genes have been revealed following cortical stroke in rats [20, 21]. The general pattern is that growth inhibitory proteins (e.g., Nogo) are inhibited for several weeks after stroke but thereafter begin to show increased expression. Conversely, growth-promoting genes, including Gap-43, are highly expressed in the first days after stroke. This pattern of post-stroke gene expression is suggestive of a ‘permissive neural niche’ for stroke recovery in the first few weeks after injury [20]. While the experience of stroke patients varies considerably depending on injury severity, disease comorbidity, staffing, and resource issues, it is the case that in the first few weeks after stroke, many patients are alone as much as 60% of their time and inactive during 75% of their waking hours [22]. Except for the period allocated for therapy sessions, the lack of stimulation, exercise, and socialization is striking. Further, current rehabilitation practice is often not early, nor intense, and most clinical trials are conducted in the chronic phase (i.e., >6 months) of stroke recovery [23].

Enriched Rehabilitation and Stroke Recovery

Many of the reparative gene programs initiated by stroke are profoundly affected by behavioral and environmental experience, such as housing animals in enriched environments (large cages with novel objects to provide exercise, sensory-motor, social and cognitive stimulation) or providing running exercise [24, 27, 28]. For example, environmental enrichment, like exercise, increases levels of growth factors, neurogenesis, and angiogenesis [28–31]. While many studies have shown that environmental enrichment improves motor recovery after stroke [27], the benefits are primarily seen on gross tests of sensory-motor function such as rotarod or neurologic test batteries and not tests of reaching that require fine motor skills [4, 32]. Accordingly, we developed a rehabilitation paradigm, termed enriched rehabilitation (ER) that combines the social, sensory-motor, and cognitive stimulation inherent to environmental enrichment with daily reach training in order to provide task-specific therapy of the affected limb, following forelimb cortical stroke in rats (fig. 1a–c). This paradigm has been successfully used to improve recovery of skilled reaching in rats following different forms of ischemic and hemorrhagic stroke [33–35].

Fig. 1. Enriched rehabilitation paradigm and the critical period of post-stroke rehabilitation. After stroke, skilled use of the affected hand (or paw) is highly resistant to recovery in both humans and animals. In rats, enriched rehabilitation produces substantial improvements in the recovery of skilled reaching [25, 34–36, 67]. The protocol consists of enriched housing (**a**) and/or running exercise (**b**) in combination with several hours of daily reach training (**c**) of the impaired limb. Rats are housed in enriched environments starting 5–14 days after stroke (**d**). The timing of rehabilitation is designed to optimally engage neuroplasticity processes during the critical period of the early post-stroke

recovery phase, during which a sustained upregulation of growth-promoting genes predominates (solid red line in part **d**). Most growth-inhibitory genes (solid green line) tend to be upregulated gradually, several weeks after stroke, toward the end of the critical period of stroke recovery. A few growth-promoting and growth-inhibiting genes are transiently upregulated (dashed lines) in the early and mid post-stroke recovery period. This critical period is observed in animal studies and might be different in the case of human stroke, where spontaneous recovery can extend for the first 90 days after injury. Data in part **d** is adapted from [20, 21]. Figure modified from [4].

Rehabilitation: Timing and the Concept of Critical Periods

In addition to improving behavioral recovery, a key effect of ER is that it increases dendritic length and complexity of layer V motor neurons in the stroke-damaged brain [25], suggesting that this structural modification may be contributing to the improved outcome. In support of this notion are animal studies showing that delayed initiation of ER until 30 days after stroke does not result in improved functional recovery nor does it alter layer V dendrites. These findings suggest there is ‘critical or sensitive period’ lasting several weeks after stroke when brain plasticity processes (fig. 1d) are optimally responsive to rehabilitation [4, 36]. Interestingly, this critical period coincides with the rising waves of growth-promoting genes that appear to peak 30 days after stroke [20]. While there is general consensus that post-stroke rehabilitation should be started sooner rather than later, there is no agreement as to exactly when it should start or when it is too late to achieve substantial benefit [23, 37, 38]. Animal studies, including some of our own research [4, 23, 36], have helped guide clinical studies including the recent EXPLICIT stroke trial evaluating early constraint-induced movement therapy [39], although an earlier constraint therapy study reported negative [40] outcomes. However, results may be influenced not only by timing, but also by the type and intensity of the intervention since early aphasia therapy [41] and early physical activity [42, 43] are associated with better outcomes.

Brain Derived Neurotrophic Factor and Stroke Recovery

Brain Derived Neurotrophic Factor (BDNF) is the most highly expressed growth factor in the human brain and plays an essential role in neuroplasticity in both the intact and the damaged brain by increasing neuronal survival, synaptogenesis, angiogenesis, and neurogenesis [44]. Administration of BDNF in shortly after stroke reduces injury [45], whereas delayed administration is associated with improved sensory-motor recovery [46, 47]. Our laboratory has investigated the possible role of BDNF in mediating the behavioral recovery induced by rehabilitation. Notably, blocking BDNF in the rat negates the benefits of rehabilitation-induced recovery after sensory-motor cortex stroke [48], while selective blockade of BDNF signaling in the peri-infarct cortex attenuates glutamate, AMPAR-mediated spontaneous motor recovery

in mice subjected to photothrombotic stroke [49]. In related work [34], it was found that a critical amount or ‘threshold’ of post-stroke reaching rehabilitation must be met to obtain functional recovery. Animals exceeding this threshold exhibited forelimb recovery and showed significant increases in motor cortex BDNF levels, while rats receiving less rehabilitation did not recover and BDNF levels remained at control levels. These findings clearly indicate that BDNF plays a key role in post-stroke rehabilitation and consequently BDNF may be an important target to enhance recovery in stroke patients.

Stem Cells and Stroke Recovery

The identification of at least two neurogenic zones in the rodent and human brain created optimism for brain repair and stroke recovery because neural stem cells and their progenitors, collectively termed neural precursor cells (NPCs), were observed to migrate toward an injury site [50–52]. Direct delivery of NPCs [53–56] or mobilization of endogenous NPCs with growth factors or drugs [57, 58] improves recovery after stroke but in most of these studies, the behavioral effects are rather small. Mesenchymal stem cells appear to be especially effective in restoring function after focal stroke. Indeed, in one recent review, it was noted that approximately 95% of studies reported improved functional outcomes [59]. However, similar impressive outcomes were noted in early neuroprotection reviews, so a more cautious and critical assessment of stem cell studies is clearly warranted. For example, many of the mesenchymal stem cell studies used rather crude neurological test scores and simple balance tests that tend to resolve rather quickly without intervention [4]. Thus, it will be important to demonstrate that stem cell therapies are effective with more demanding behavioral tests where spontaneous recovery is limited.

New Approaches to Brain Repair – Combination Therapies

Increasing evidence indicates that the causes of ischemic cell death are *multi-factorial* [3]. Furthermore, the brain responds to vascular insults by marshalling a wide array of self-protective mechanisms [3, 60] that stand in marked contrast to the *single-target* approach commonly employed in neuroprotection [61]. When interventions (e.g., hypothermia) target multiple, instead of single mechanisms, the results are impressive [3, 62]. Similarly,

a more effective approach might be to use a ‘cocktail’ intervention to exploit multiple complementary neuroplastic mechanisms to restore post-stroke recovery. For example, each of the individual elements of enriched rehabilitation (exercise, socialization, cognitive stimulation, reach training) has some therapeutic potential but collectively provide a powerful intervention by acting upon multiple complementary processes of neuroplasticity (e.g., upregulation of growth factors, angiogenesis, dendritic sprouting) [27, 63]. This was illustrated in a recent experiment showing that ER, but not enriched environments or reach training alone, strongly activated neurons in layer II and III peri-infarct cortex [64], a region thought to be critical for recovery of sensory-motor function [4, 65, 66]. These findings indicate that a *combination* of environmental enrichment and task-specific rehabilitation targeting the main functional deficit (i.e., skilled reaching) most effectively increases neuronal activity around the lesion. Similarly, in more recent work, we found that serial administration of epidermal growth factor and erythropoietin induced mobilization of the endogenous NPC pool and resulted in significantly faster and more complete recovery after cortical stroke in rats when it was combined with ER [67]. This is the first demonstration that stem cell therapy produces an additive benefit in stroke recovery beyond what can be achieved with an optimized rehabilitation paradigm. This is important because stem cell therapies (or other interventions) will likely not be employed unless they provide benefit in beyond what is already achieved through current best practice (i.e., rehabilitation).

Designing preclinical and clinical studies using combination therapies is more complicated and costly than

traditional single intervention studies. Nonetheless, the efficacy of single interventions in stroke is abysmal and sooner or later, this ‘silver bullet’ approach must be abandoned [3]. Adapting preclinical enrichment paradigms to the clinic is possible and early results with enriched rehabilitation in stroke patients are encouraging [68]. Clearly the post-stroke environment has the potential to significantly impact the brain’s intrinsic restorative programs and in so doing profoundly affect stroke recovery. Indeed, enriched rehabilitation, unlike many other preclinical post-stroke interventions, has shown consistent benefits across laboratories and stroke models [25, 35, 69].

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Disclosure Statement

No conflicts of interest.

Ethics

All research conducted by the authors described in this article conformed to the guidelines established by the Canadian Council on Animal Care and were approved by the institutional animal care committees of Memorial University of Newfoundland and the University of Ottawa.

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