POST-STROKE AUGMENTATION OF REHABILITATION-MEDIATED FUNCTIONAL RECOVERY WITH GROWTH FACTOR ADMINISTRATION

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By

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Abstract

Behavioural rehabilitation is the only treatment option for chronic stroke deficits. Unfortunately, even lengthy rehabilitation often provides incomplete recovery. This study used an animal model of stroke that incorporates key features of sensorimotor impairment commonly observed in stroke patients. A novel combination of growth factor administration and rehabilitation therapy was employed to facilitate functional recovery in this model.

Sprague-Dawley rats received a stroke via injection of endothelin-1 at two sites in the sensorimotor cortex. This was followed by either a 2-week infusion of epidermal growth factor (EGF) and erythropoietin (EPO) or artificial cerebrospinal fluid (aCSF). Two weeks post-ischemia, animals began either a 6-week enriched rehabilitation program or standard housing treatment: (1) EGF/EPO + rehab; (2) aCSF + rehab; (3) EGF/EPO + no rehab; and (4) aCSF + no rehab. Functional assessments were performed pre- and post-ischemia and after 14, 28, and 42 days of rehabilitation thereafter (approximately every 3 weeks) using the Montoya staircase reaching task, beam traversing and cylinder test of forelimb asymmetry.

The combination of EGF/EPO + rehab led to a significant acceleration in recovery on the Montoya staircase reaching task after only 2 weeks of therapy compared to rehabilitation-alone. Although the combination of EGF/EPO + rehab resulted in accelerated recovery, animals exposed to rehabilitation-alone recovered to a similar extent after 6 weeks of therapy. This effect was observed in both the staircase and beam traversing tasks where animals that received rehabilitation recovered to a significantly greater extent than standard-housed animals.

Combining behavioural rehabilitation with growth factors that promote endogenous stem cell mobilization may accelerate recovery beyond that of rehabilitation alone. This has the potential to reduce the length of rehabilitation necessary to recover from stroke deficits.

Acknowledgements

Without the tireless dedication of many people, this thesis would not be possible. I would like to sincerely thank Dr. Dale Corbett for the guidance, encouragement and support that he has given me both inside the lab and out. The opportunities and challenges that he presented me throughout my studies will shape the approach I take to scientific thought for the rest of my life.

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List of Abbreviations

aCSF – artificial cerebrospinal fluid

ANOVA – analysis of variance

AP – anteroposterior

Ca^{2+} – calcium ion

DV – dorsoventral

EGF – epidermal growth factor

EPO – erythropoietin

ET-1 – endothelin-1

FGF-2 – basic fibroblast growth factor

MCA – middle cerebral artery

MCAo – middle cerebral artery occlusion

ML – mediolateral

NMDA – N-methyl-D-aspartic acid

PFA – paraformaldehyde

REGW-F – Ryan-Einot-Gabriel-Welsch F

SVZ – sub-ventricular zone

tPA – tissue plasminogen activator

VEGF – vascular endothelial growth factor

Introduction

1.1 Overview of Stroke Statistics

Stroke is the third leading cause of death in Canada, resulting in approximately 15,000 deaths a year and over 30,000 hospitalizations (Johansen et al., 2006; Canadian Institute for Health Information, 2009). This results in annual losses of over $3 billion per year to the Canadian economy in both direct health care costs for patients and lost economic output due to disability (Public Health Agency of Canada, 2002). In addition to these severe, or overt strokes that demand immediate medical attention, it is estimated that the incidence of smaller, or covert strokes may be 10-20 times more frequent (Longstreth, 1998). Covert strokes may result in deficits that are initially more subtle but certainly increase the risk of suffering a subsequent, severe stroke by a factor of five (Vermeer et al., 2003). This indicates that stroke is potentially a much more serious problem than currently recognized.

Some consider the most devastating effects of stroke to be the permanent motor disabilities that commonly result from this condition. Unfortunately, current treatment methods provide only incomplete recovery, and require intensive treatment periods in order to obtain significant clinical gains. For this reason, research into improved treatment techniques for stroke recovery is of the utmost importance in order to assist patients in returning to their normal lives. The current thesis aims to contribute to this area by assessing the ability of a novel combination of growth factor treatment and behavioural rehabilitation to accelerate the rate of motor recovery in an animal model of ischemic stroke.

1.2 Pathophysiology of Ischemic Stroke

Stroke is a sudden disruption of extra- or intra-cranial blood flow resulting from one of two conditions. The first, known as hemorrhagic stroke, occurs when blood vessels rupture, causing a leakage of blood into brain tissue. The second, and most common type of stroke (~80% of cases), is referred to as ischemic stroke; a condition that develops from a blockage of one or more of the blood vessels in the brain. The resulting drastic reduction of oxygen and glucose to regions downstream of the blockade quickly disrupts the local neuronal populations' ability to maintain their ionic gradients (Martin et al., 1994). Membrane potential is rapidly lost, causing a depolarization of neurons that leads to a massive influx of calcium ions (Ca^{2+}) and subsequent release of glutamate into the extracellular space (Katsura et al., 1994). Excessive stimulation of N-methyl-D-aspartic acid (NMDA) and glutamate receptors by this excessive extracellular glutamate causes a further influx of Ca^{2+} (McCulloch et al., 1993). This initiates a cascade of nuclear and cytoplasmic events that ultimately lead to necrosis and apoptosis of neurons in the ischemic core (Furukawa, et al., 1997; Dirnagl et al., 1999). Between the ischemic core and normal brain regions is an area with only partially disrupted ionic balance and reduced blood flow, known as the peri-infarct region. It is thought that if tissue in this area can be spared from cell death, stroke-related impairment will be reduced, making it an important research target in attempts to maximize recovery in the post-stroke period (De Keyser et al., 1999).

1.3 Animal Models of Ischemic Stroke

In order to develop new methods for treating stroke-related impairments, animal models are necessary. These models allow experimental manipulation and assessment of novel treatments and enable the study of underlying cellular mechanisms responsible for enhanced functional recovery. A number of different species have been used to model many of the physical and cognitive deficits that can be caused by stroke (Corbett & Nurse, 1998). However, rats are the most commonly used animal for studying functional impairment and rehabilitation after stroke because they offer the advantages of being relatively inexpensive, having a cerebrovascular anatomy that is similar to humans and exhibiting limb movement and skilled reaching abilities that resemble those of humans (Whishaw et al., 1992). These benefits enable the study of post-stroke recovery patterns and the effects of novel interventions using a number of different methods of producing stroke.

One of the most widely used models of producing focal ischemic stroke in the rat has been the middle cerebral artery occlusion (MCAo) method. Occlusion of the middle cerebral artery (MCA) can be produced using a variety of techniques. Traditionally, the most commonly used method involves a transient occlusion whereby a sterile suture is inserted through the external carotid artery, passed along the internal carotid artery and lodged at the junction of the anterior and middle cerebral arteries (Longa et al., 1989). Typically, this suture is left in place for 60, 90, or 120 minutes. Suture occlusions of longer than 60 minutes can result in hypothalamic damage (Garcia et al., 1995). This triggers a hyperthermic response in the animal that exacerbates cell death and does not usually occur in human stroke (Reglodi et al., 2000). Other models of MCAo have been

developed that avoid damage to deep brain structures such as the thalamus and hypothalamus (Carmichael et al., 2005). These MCA models involve surgical separation of the parotid gland and temporalis muscle and a craniotomy over the MCA (Tamura et al., 1981). Occlusion distal or proximal to the branching of the striatal arteries will result in purely cortical or cortical plus striatal injury, respectively. This prevents the hyperthermic response observed in suture models of MCAo, but is a more challenging technique and requires a much more invasive surgery (Yamashita et al., 1997). Both the distal and proximal MCAo methods, as well as the intraluminal suture model, create a large and variable injury in rodents. These infarcts are of a magnitude not usually observed in human stroke survivors and thus, MCAo methods may more accurately model malignant infarction than typical human ischemia from which recovery is possible (Carmichael, 2005). A final modification of the MCAo model is to inject blood clots or microspheres into the artery (Miyake et al., 1993; Zhang et al., 1997). These injections produce multifocal lesions throughout the brain that mimic the scope of human infarctions, however, these infarcts are of variable size and location, making evaluation of therapies that rely on damage in a particular brain region extremely difficult with this method (Beech et al., 2001). In all of the MCAo models mentioned, but particularly the intraluminal suture model, the forelimb motor cortex is often spared (Carmichael, 2005; Windle et al., 2006), rendering this model less useful for studies involving rehabilitation of post-stroke forelimb and grasping function.

In order to produce smaller, more focal lesions than with traditional MCAo models, other techniques have been developed, including photothrombosis (Watson et al., 1985). With this model, the area of the brain to be lesioned is exposed via craniotomy.

Following the craniotomy, a light-reactive dye, Rose Bengal, is injected and once the dye has had a chance to circulate throughout the body a laser light source is directed at the prospective lesion site in order to excite the photosensitive dye. This produces local singlet oxygen that causes free radical damage to all blood vessels in the area of illumination. The resultant endothelial damage then initiates focal platelet aggregation and a clotting response that blocks regional blood flow and causes localized ischemic damage (Watson et al., 1987). Although this technique allows for the creation of extremely controlled lesions, all vessels and capillary beds in the ischemic region are permanently compromised without further surgical intervention (Watson et al., 2002; Yao et al., 2003). The gradual reperfusion of the ischemic site that is typically seen in human stroke is not possible with this model.

Another method of producing small, focal stroke lesions in a targeted brain region is through local injection of the vasoconstrictor peptide, endothelin-1 (ET-1) (Fuxe et al., 1997). This peptide activates voltage-dependent Ca^{2+} channels in the vascular endothelium, resulting in a reduction of arterial size that subsequently restricts blood flow to adjacent tissue (Yanagisawa, 1988). This method is more advantageous than photothrombosis because reperfusion of affected areas occurs gradually over time (Biernaskie et al., 2001; Windle et al., 2006). Reperfusion also naturally occurs in most cases of human stroke, making this an excellent model for experimental stroke research (Carmichael, 2005). Selecting a good model for inducing stroke in animals is important for ensuring that research findings will be applicable when testing transitions into a clinical setting.

1.4 Neuroprotective Strategies

To date, only two treatments have been successfully translated from basic animal research to provide neuroprotection following ischemic stroke in humans. The first involves administration of the thrombolytic enzyme known as tissue plasminogen activator (tPA) (NINDS, 1995). This enzyme re-establishes perfusion to ischemic brain regions by breaking down blood clots that are impeding normal flow. Successful administration within 4.5 hours of ischemic stroke onset results in improved functional outcome and a reduction in pathological tissue damage (Heiss et al., 1998; Albers et al., 2002). However, if the patient does not present to the hospital within this time window, of which the majority do not (67.6%; Nadeau et al., 2005), administration of tPA does not result in significant functional benefits and has been shown to increase the acute mortality rate of patients (NINDS, 1995).

The second method of reducing stroke damage is therapeutic application of hypothermia. Using animal models of ischemia, it has been demonstrated that lowering the core body temperature can both improve functional outcome and decrease cell death (Colbourne & Corbett, 1994; Colbourne & Corbett, 1995). The underlying protective mechanisms of hypothermia are thought to involve a reduction of the metabolic rate, modulating Ca^{2+} signalling and glutamate receptor activation, causing an attenuation of the physiological cascade that normally results in ischemic damage (Lazzaro & Prabhakaran, 2008). Despite the promising results displayed by hypothermic treatment, a lack of clinical guidelines for its use in stroke prevents its widespread use in acute stroke care (Lyden et al., 2006). Because the majority of stroke patients are not able to receive

tPA treatment or hypothermia in the time that these interventions would be most beneficial, most patients develop chronic functional impairments related to their stroke. As a result, research into alternative means of treating these long-term impairments is necessary.

1.5 Recovery from Chronic Stroke Impairment

Following brain damage, many cellular processes are initiated in an attempt to stabilize the ischemic core, promote neuroplastic change and growth of surviving neurons, and reinforce newly formed connections. Migration of neuroblasts and astrocytes to the site of injury takes place quickly after damage has occurred (Jin et al., 2003; Goings et al., 2004). Many of these cells differentiate into astrocytes, which then secrete a variety of beneficial neurotrophic factors such as brain-derived neurotrophic factor and neuronal growth factor into surrounding tissue (Ridet et al., 1997; Chen & Swanson, 2003). These growth factors augment the ability of neuronal tissue in the peri-infarct region to undergo neuroplastic change, providing an important opportunity to re-establish disrupted cortical connections and thus restore lost motor function (Carmichael, 2006). Despite these favourable conditions for recovery of motor function, only small improvements in outcome occur spontaneously. Attaining significant levels of motor recovery requires extraneous behavioural intervention (Biernaskie & Corbett, 2001).

1.6 Enhancing Motor Recovery with Rehabilitation

Stimulating neuroplasticity mechanisms in peri-infarct regions provides the capability for the cortical remapping necessary to improve functional outcomes beyond

limited spontaneous recovery. Clinical motor rehabilitation capitalizes upon the reorganizational capacity of the injured brain by using regular and repeated stimulation of stroke-impaired limbs to stimulate cortical reorganization (Hodics et al., 2006).

Achieving the optimal results from a motor rehabilitation program requires several conditions to be met. In order to achieve maximal recovery of complex motor movements, rehabilitative therapy must simulate the motor patterns required in the specific task for which rehabilitation is desired (Richards et al., 1993). Task-specific therapies enable recruitment of non-affected brain regions that are adjacent to cortical damage to restore lost function (Nudo et al., 1996). Clinical studies have also demonstrated the importance of task-specific therapy in performance of experimental outcome measures and normal life activities (Langhammer & Stanghelle, 2000; Blennerhassett & Dite, 2004). In addition to therapies being task-specific, both animal and human studies agree that the greatest gains in motor function are achieved when rehabilitation is administered soon following stroke (Biernaskie et al., 2004; Salter et al., 2006). Delaying rehabilitation one month after stroke results in a loss of cortical representation, making recovery of function difficult (Barbay et al., 2006). Along with administration of task-specific therapy early after stroke, ensuring that rehabilitation provides challenging and intense motor stimulation is also important for maximizing functional outcome (Kwakkel et al., 2004; Birkenmeier et al., 2010). It is thought that greater recovery is realized when more repetitions of rehabilitative exercises are performed in both animal and clinical studies. This has led to a theory that motor benefits are not gained until a threshold of duration and intensity of rehabilitative therapy has been surpassed (Han et al., 2008; MacLellan et al., 2011).

Early, intensive, task-specific therapy is important for maximizing motor recovery following stroke because this combination takes advantage of the 'primed' peri-infarct tissue for neuroplastic change resulting from the milieu of growth factors present in damaged tissue that promote growth of new neural connections (Carmichael et al., 2006). Animal studies have demonstrated that the benefits of these growth factors can be further augmented by placing animals in enriched housing environments, leading to an upregulation of growth factors and neurogenesis (Falkenberg et al., 1992; Bruel-Jungerman et al., 2005; Gelfo et al., 2010). High intensity rehabilitation is thought to strengthen newly formed connections and task-specific rehabilitation supports repeated use of disrupted neural circuits, causing an increase of their cortical representation as the brain reorganizes around infarcted tissue (Nudo et al., 1996; Hodics et al., 2006).

Although self-repair mechanisms that can be enhanced by rehabilitation exist in the brain, this treatment is not without its limitations. Functional gains that are made come at the cost of large investments of time and effort on the part of both the patient and medical personnel in rehabilitation. Achieving maximal levels of recovery requires months of therapy and patients' abilities often plateau below their pre-stroke level of function (Yagura et al., 2003). In order to overcome these limitations and enable greater recovery of function at an accelerated rate, it may be necessary to augment neuroplasticity and promote replacement of lost tissue via exogenous therapies. This may be possible through supplementary administration of growth factors in combination with conventional rehabilitation techniques.

1.7 Growth Factor Infusion and Stroke

Administration and endogenous upregulation of growth factors has garnered much interest in attempting to provide neuroprotection and improve recovery of motor function following stroke. A wide variety of growth factors have been tested in this regard, including basic fibroblast growth factor (FGF-2), bone morphogenetic protein-7, vascular endothelial growth factor (VEGF), and granulocyte colony stimulating factor (Fisher et al., 1995; Zhang et al., 2000; Chang et al., 2003; Shyu et al., 2004). However, while these studies demonstrate that simple administration of growth factors conveys moderate benefit in reducing stroke-related damage in animals, functional improvement is limited to gross motor movements. Two growth factors that have shown particular promise in aiding in stroke recovery are epidermal growth factor (EGF) and erythropoietin (EPO).

EGF is one of the most powerful mitogenic proteins in the human body. This protein has been shown to increase the proliferation of neural stem cells from the sub-ventricular zone (SVZ) in the brain (Reynolds &Weiss, 1992). These cells are of particular interest in post-stroke recovery, because cells from this region naturally migrate to the site of injury following damage to the brain (Jin et al., 2003; Goings et al., 2004). If this process is impeded, post-stroke impairment is exacerbated, implicating these cells in motor recovery processes (Tsai et al., 2006). In addition to aiding proliferation of neural stem cells in the SVZ, EGF induces migration of radial glia to the site of cortical damage, potentially enhancing the ability of cells to migrate to the site of injury (Gregg & Weiss, 2003). EGF also has a neuroprotective effect on cells undergoing traumatic events by inhibiting apoptotic processes (Liu et al., 2006). Increasing the proliferation of cells that are migrating to the ischemic site may enhance neural repair by replacing lost tissue,

or supporting the reorganization of surviving tissue through local secretion of additional growth factors.

EPO is an angiogenic protein that has been found to increase reperfusion of infarcted areas by promoting vascular growth (Carlini et al., 1995; Chong et al., 2002). In vivo administration of EPO promotes neuroblast migration to the ischemic site (Wang et al., 2004). Continued exposure to EPO can then induce differentiation of neuroblasts into a neuronal phenotype (Shingo et al., 2001). EPO also exerts a positive effect on astrocytes in the ischemic site by increasing expression of other beneficial growth factors such as vascular endothelial growth factor and brain derived neurotrophic factor (Wang et al., 2004). Deposition of proteins that exert an inhibitory effect on neural growth and plasticity such as chondroitin sulfate proteoglycans are downregulated by EPO administration (Vitellaro-Zuccarello et al., 2008). By increasing the population of viable cells and expression of growth factors in the ischemic core and peri-infarct region, post-stroke administration of EPO may enhance tissue regeneration and neuroplasticity.

Serial administration of EGF and EPO in rats has been shown to improve post-stroke outcome on several measures of motor recovery beyond what is possible with either factor alone or control infusion (Kolb et al., 2007). Augmenting neuroplasticity in this fashion may enable rehabilitation-mediated functional recovery to occur at an accelerated rate, to a level that is not possible with rehabilitation alone.

1.8 Overview of Experiment

The present study was the first to assess the combinatory effects of growth factor administration and enriched rehabilitation on post-stroke recovery of motor function. As

previously mentioned, administration of EGF and EPO may have beneficial effects on stroke outcome, potentially through migration of neuroblasts or a growth factor repository in the site of ischemic injury and peri-infarct cortex. Subsequent administration of conventional rehabilitation techniques may capitalize on the beneficial properties of these events, leading to a faster and larger reorganization of surviving cortical tissue involved in the relevant motor pathways. For this reason we hypothesized that administering a combination of EGF, EPO and a subsequent rehabilitative treatment regime following forelimb sensorimotor cortex ischemia would result in accelerated functional improvements than with either rehabilitation- or EGF and EPO-alone. Additionally, we believed that the combination of EGF/EPO with rehabilitation would produce a synergistic effect, leading to a higher plateau of motor performance than with either therapy alone.

Methods

2.1 Subjects

Sixty-seven male Sprague-Dawley rats, (Charles River Laboratories, Montreal, Quebec, Canada) weighing 300-325 grams upon arrival, were used in this study. Animals were handled for several minutes a day over the first four days after arrival and pair-housed in Plexiglas© cages on a 12:12 hour reverse light/dark cycle with lights off at 8:00. Food and water were accessible *ad libitum* except during behavioural training and testing periods, when animals were restricted to 90% of their free-feeding weight. All procedures and testing were performed during the dark cycle. Data from 50 animals, divided among four groups, were used in the final behavioural analyses.

2.2 Experimental Conditions

The experimental design of the current study consisted of a 2x2 matrix with osmotic pump infusion (pump) and rehabilitation therapy (rehab) as independent variables. Following post-stroke testing, animals were pseudo-randomized into 1 of 2 conditions (matched for performance on staircase task): consecutive infusion of EGF and EPO or two infusions of artificial cerebrospinal fluid (aCSF). Both groups were then further divided so that animals would be exposed to either enriched rehabilitation treatment (Rehab), or standard housing (No Rehab). The resulting experimental design consisted of 4 conditions: Rehab + EGF/EPO (n = 13), Rehab + aCSF (n = 12), No Rehab + EGF/EPO (n = 12), No Rehab + aCSF (n = 13). All procedures were approved by the Memorial University of Newfoundland Animal Care Committee and comply with regulations set by the Canadian Council of Animal Care.

2.3 Behavioural Training and Testing

2.3.1 Staircase Test of Skilled Reaching Performance

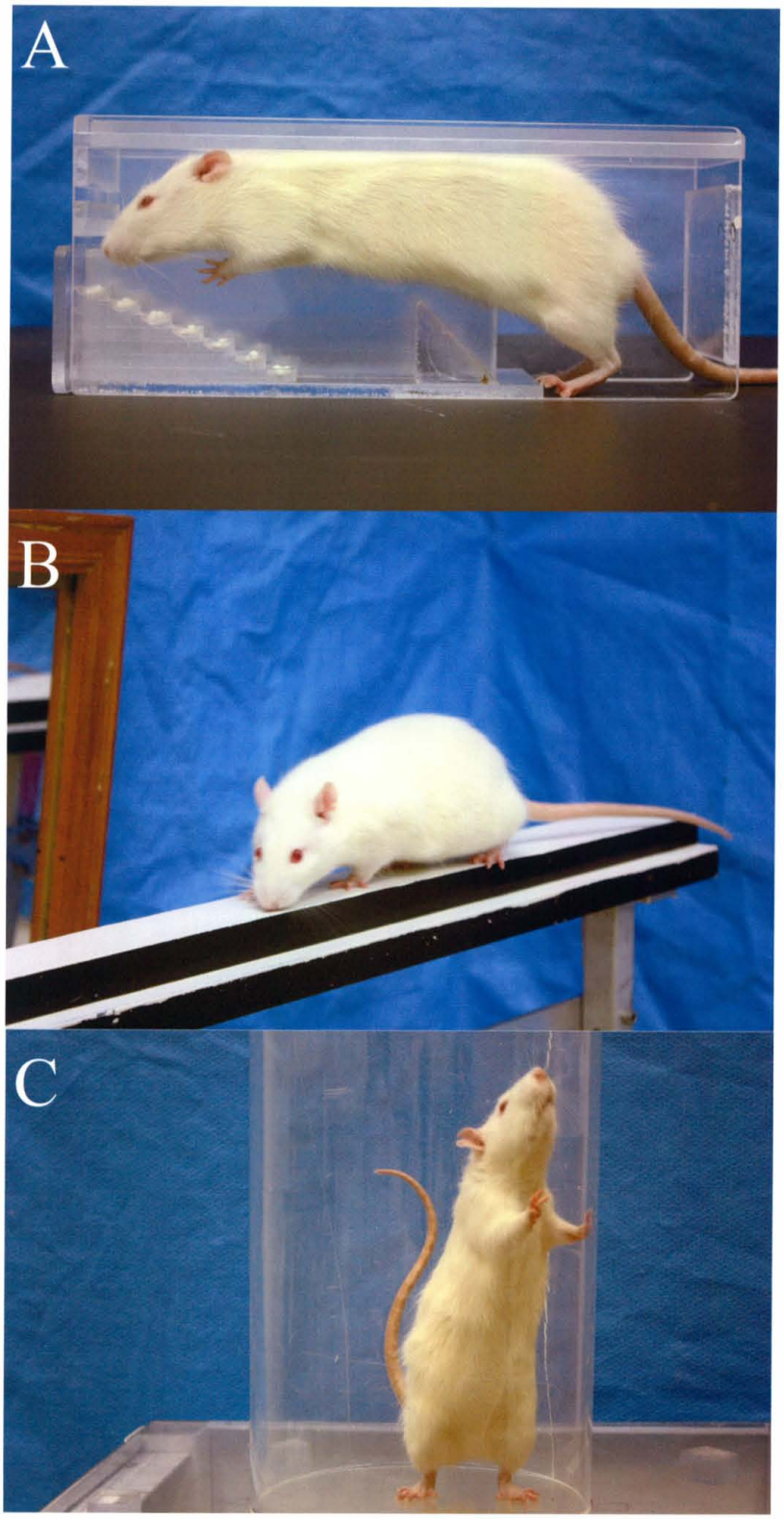
The staircase test enables assessment of forelimb reaching and grasping capabilities providing a means to evaluate the severity of animals' forelimb motor deficits following stroke (Montoya et al., 1991). Animals rest on a central platform in a Plexiglas box and are able to reach for 45 mg sugar pellets (TestDiet, Richmond, Indiana, USA) on two staircases situated on each side of their body (Figure 1A). The pellets on each staircase are only accessible by the ipsilateral limb and are composed of seven levels (containing three pellets each) that are progressively more distant from the animal. Acquiring the food reward requires arm extension and fine digit manipulation and increases in difficulty with descending steps. Forelimb reaching ability is measured by the accuracy of obtaining pellets in a given trial.

Prior to stroke, all animals were trained in the staircase apparatus twice per day for 14 consecutive days. Each trial lasted for 15 minutes and was separated by a minimum of four hours. Performance across the final two days was collapsed and animals failing to retrieve a minimum of 12 out of the 21 possible pellets with the dominant paw (average was 17.9 pellets), with a standard deviation of less than two pellets, were eliminated from the study ($n = 5$). Performance at this time point was used as a measure of each animal's baseline reaching ability.

The first post-stroke assessment occurred five days after surgery in order to assess the functional impairment of each animal. Animals with the ability to obtain greater than 60% of their baseline number of pellets were excluded from the study ($n = 12$).

Figure 1. Battery of behavioural tests for functional motor performance.

(A) Lateral view of the Montoya staircase reaching task. (B) Beam-traversing task. A step was scored as a fault if the pad of the foot slipped from the top (white) level of the beam. (C) Cylinder test. Forepaw use was measured by filming from below the animal (not shown).

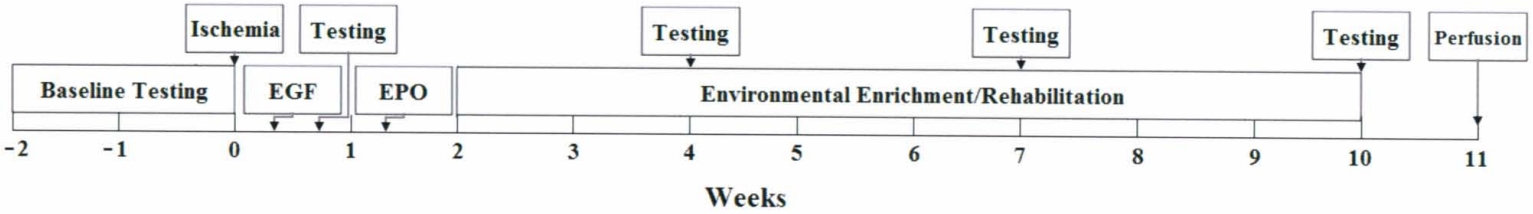

A
B
C

This ensured that the experimental population consisted of animals with moderate to severe stroke for whom therapy is required in order to significantly recover from stroke-induced impairment. Excluded animals were still housed and tested alongside the remaining animals to avoid altering cage dynamics. Throughout the enriched rehabilitation treatment, animals were assessed after each fourteen days of rehabilitation (approximately every 3 weeks) in the behavioural assay (Figure 2). At each post-stroke time point, animals' reaching abilities were assessed in 6 trials over 3 consecutive days (2 trials/day for 15 minutes). Data from the first day of testing (first 2 trials) were not analyzed, as the purpose of these trials was to allow the animals to reacclimatize to the testing environment.

2.3.2 Beam-Traversing Performance

The beam-traversing test challenges an animal's ability to cross a tapered beam (length, 160 cm; widest portion, 6 cm; narrowest portion, 2 cm) elevated 75 cm above the ground in order to reach a darkened chamber at the narrow end (Figure 1B). A 1 cm wide ledge (1 cm below upper level of beam) was positioned on both sides of the beam to help prevent the animal from falling from the beam when its foot slipped. Animals naturally attempt to stay on top of the upper level of the beam (Kolb & Whishaw, 1983). Correct performance of this task requires progressively more coordinated and skilled paw placement in order to avoid slipping to the safety ledge as the beam tapers. Trials were video recorded and performance was measured for each limb by calculating the proportion of steps in which the pad of the foot slipped from the top of the beam relative to the total number of steps.

Figure 2. Timeline of experimental procedure.


Ischemia
Testing
Testing
Testing
Testing
Perfusion
Baseline Testing
EGF
EPO
Environmental Enrichment/Rehabilitation
-2
-1
0
1
2
3
4
5
6
7
8
9
10
11
Weeks

Prior to baseline testing, animals were trained to cross the beam to a darkened chamber placed at the narrow end. Rats were required to repeatedly cross the beam until they were able to traverse from the widest portion of the beam to the goal box without pausing or noticeably slipping on four consecutive trials. Testing occurred pre- and post-stroke as well as bi-weekly throughout rehabilitation treatment. On each test day, animals were required to traverse the beam four times and data from all trials were averaged. The percentage of successful steps was calculated as: (1 – [foot faults/total steps]) * 100.

2.3.3 Cylinder Test of Forelimb Asymmetry

The cylinder test measures the portion of forelimb use for postural support during rearing (Jones & Schallert, 1994; Schallert et al., 2000). Animals were placed in a Plexiglas cylinder (20 cm diameter) on a glass tabletop and videotaped from below (Figure 1C). Each trial continued until the subject completed a minimum of 20 independent rears and wall contacts. The number of contacts with each paw was analyzed and use of the limb contralateral to the stroke was calculated as: ([contralateral contacts + ½ bilateral contacts]/total contacts) * 100. Testing occurred pre- and post-stroke as well as bi-weekly throughout rehabilitation treatment.

2.4 Surgical Procedures

2.4.1 Focal Ischemia

Following baseline behavioural testing, rats were anesthetised with isoflurane (3% induction, 1.5% maintenance; CDMV Canada, St-Hyacinth, Quebec, Canada) in 100% O_2 (1.6 L/min). Animals were secured in a stereotaxic frame and received a 0.2 mL scalp

subcutaneous injection of 1% lidocaine (AstraZeneca, Mississauga, Ontario, Canada). The midline of the scalp was incised and three holes were drilled in the skull over the forelimb sensorimotor cortex, contralateral to the paw of best performance in the baseline staircase reaching test. At two of these sites, 2 μL of endothelin-1 (ET-1; 400 pmol/μL; Calbiochem, La Jolla, California, USA) was injected into the brain at the following co-ordinates (relative to bregma): 0.0 mm anteroposterior (AP), ±2.5 mm mediolateral (ML), -2.5 mm dorsoventral (DV); +2.3 mm AP, ±2.5 mm ML, -2.5 mm DV. The third drill hole located at -0.5 mm AP, ±1.5 mm ML was used for cannulation of the lateral ventricle during a subsequent surgery. Rectal temperature was maintained at a minimum of 36.5°C with a homeothermic blanket (Harvard Apparatus, Saint-Laurent, Quebec, Canada) throughout the surgery. After ET-1 injection was complete, the incision site was sutured and topical 2% Xylocaine (AstraZeneca, Mississauga, Ontario, Canada) was applied. Anesthesia was then discontinued and animals were placed in a cage on a heated blanket until consciousness and normal mobility were restored.

2.4.2 Osmotic Mini-Pump Implantation

Three days after stroke induction, animals were re-anesthetized and their scalps re-incised. A 5 mm infusion cannula was inserted into the lateral ventricle at -0.5 mm AP, ±1.5 mm ML relative to bregma and secured in place with cyanoacrylate glue (Loctite, Mississauga, Ontario, Canada). This cannula was attached to an osmotic minipump (1.0 μL/hr, 7 days; Alzet, Cupertino, California, USA) containing either EGF (10 μg/mL) or aCSF via surgical tubing. The osmotic minipump was placed

subcutaneously between the scapulae and the scalp was sutured. Topical 2.0% Xylocaine was then administered to the incision site and anesthesia terminated.

Seven days following EGF pump implantation and cannulation, animals were re-anesthetized. A small incision was made slightly anterior to the position of the osmotic pump and the pump was removed. A new pump containing either EPO (1365 IU/mL) or aCSF was secured to the cannulised surgical tubing. The incision site was sutured, topical 2.0% Xylocaine was administered to the incision site and anesthesia terminated. All osmotic minipumps were removed after seven days.

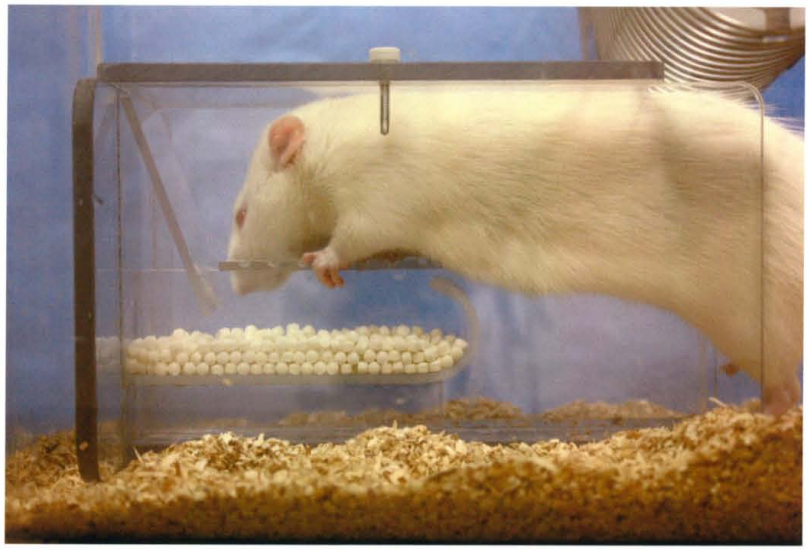
2.5 Enriched Rehabilitation

Two weeks after induction of focal ischemia, animals were pseudo-randomized to either the enriched rehabilitation (Rehab) or no-rehabilitation (No Rehab) group. Animals in the Rehab group were housed in large wire mesh cages (length, 105 cm; width, 67 cm; height, 75 cm) in groups of five or six while those in the No Rehab group were pair-housed in standard cage conditions. Wire mesh cages contained a variety of objects (platforms, ropes, tubes, ramps, balls, etc.) that were changed twice per week and placed in different locations in order to increase novelty.

Rehabilitation was received by animals housed in the enriched environment. For six hours per day, animals were removed from the enriched environment and placed in standard rat housing that contained a modified reaching box (Figure 3). This box allowed free access to an environment similar to the staircase reaching test (task-specific) in which the animal could reach for the same pellets used in the staircase, using only its impaired forelimb. The food reward was placed at a level that was only accessible

Figure 3. Lateral view of the modified reaching rehabilitation box.

Animals can freely enter the box and reach for a food reward with their impaired limb.



through making a proper reaching and grasping movement. To control for possible confounding effects of pellet consumption, animals that did not receive rehabilitation were administered a similar number of pellets (~15 g) per day in addition to their normal food. Enriched environment housing continued for eight weeks and animals received rehabilitative training five days/week except during periods of behavioural testing.

2.6 Histological Procedures

Following all behavioural assessments, animals were deeply anesthetized (5% isoflurane in O_2) and transcardially perfused with ice-cold, 0.9% heparinized saline, followed by 4% paraformaldehyde (PFA). Brains were removed and post-fixed in 4% PFA overnight at 4°C, then transferred into a 20% sucrose-phosphate buffer solution until saturated. The brains were then frozen in isopentane on dry ice, sectioned on a cryostat at 15 μm and stained with cresyl violet to assess cortical damage created by ET-1 injection. The section of maximal cortical damage was identified and severity of cortical damage was calculated as follows: 1-(area of undamaged ipsilesional cortex/area of undamaged contralesional cortex) * 100 (ImageJ 1.36b software for Mac, downloaded from the public domain, National Institutes of Health, USA, http://rsb.info.nih.gov/ij/). This generated a value for the percentage of damaged cortex in the lesioned hemisphere relative to homologous tissue in the un-lesioned hemisphere (Ploughman et al., 2005). Each animal was assigned a score on a 5-point scale that corresponded to its amount of damaged cortical tissue: 0, no ischemic damage; 1, 1-25% damage; 2, 26-50% damage; 3, 51-75% damage; 4, >75% damage.

2.7 Statistical Analysis

Statistical analyses were conducted using the statistical package for the social sciences (SPSS; v 13.0.0 Grad Pack for Mac OS X, SPSS, Chicago, IL, USA). Histological data were analyzed using the Kruskal-Wallis and Mann-Whitney U non-parametric tests. Behavioural data were analyzed using 3-way repeated measures analysis of variance (ANOVA; between-groups, Rehab, Pump; within-groups, Time) with Ryan-Einot-Gabriel-Welsch F (REGW-F) post-hoc and independent samples t-tests (Bonferonni correction) for multiple comparisons. The number of pellets retrieved during daily reaching therapy was assessed using independent samples t-test. Mauchly's test of sphericity and Levene's test for homogeneity of variance were performed for each ANOVA (where appropriate) with Huynh-Feldt and Brown-Forsythe corrections made when these conditions were not satisfied. Significance was set at $p \leq 0.05$ for all analyses and values are expressed as group means ± SEM.

Results

3.1 Behavioural Testing

One-way ANOVAs indicated that all groups suffered impairments of similar magnitude on all behavioural tasks prior to initiation of enriched rehabilitation (data not shown; $p > 0.05$). This time-point was then excluded from further analysis in order to assess treatment effects. An independent samples t-test showed that the average number of pellets acquired during daily reaching therapy was not significantly different between the Rehab + EGF/EPO and Rehab + aCSF groups ($p > 0.05$).

3.1.1 Staircase Test of Skilled Reaching Performance

A 3-way repeated measures ANOVA (between-groups: Rehab, Pump; within-groups: Time) revealed a Time X Rehab X Pump interaction ($F_{2,92}$= 4.87, $p < 0.01$). Two-way ANOVAs at each time point indicated that a significant Rehab X Pump interaction existed between groups after both 2 ($F_{3,46}$ = 3.022, $p < 0.04$) and 6 weeks ($F_{3,46}$ = 4.837, $p < 0.01$) of treatment (Figure 4A). REGW-F post hoc analysis showed that the Rehab + EGF/EPO group performed significantly better than the No Rehab + aCSF and No Rehab + EGF/EPO groups at both 2 and 6 weeks ($p < 0.05$).

In order to assess the magnitude of improvements over the course of this experiment, performance at each time point among each condition was subtracted from its post-stroke test-point (henceforth referred to as "rehab score"). REGW-F analysis indicated that after 2 weeks of treatment animals in the Rehab + EGF/EPO condition had improved significantly more on the staircase test than those in the No Rehab + EGF/EPO and No Rehab + aCSF groups ($p < 0.05$) (Figure 4B). It was not until after 6 weeks of treatment that animals in the Rehab + aCSF group showed a significant improvement over the No Rehab + EGF/EPO group ($p < 0.05$) (Figure 4C).

3.1.2 Beam-Traversing Performance

Data from the fore- and hindlimbs were averaged to provide a measure of beam-walking success for the limbs contralateral to the stroke. There was no Time X Rehab X Pump interaction ($p > 0.05$). Two-way repeated measures ANOVA indicated a Time X Rehab interaction ($F_{1.961,90.221}$= 6.467, $p < 0.01$) with animals in the Rehab condition performing significantly better than those in the No Rehab condition (Figure 5B).

Figure 4. Post-ischemic assessments of skilled reaching ability in the staircase test (mean ± SEM). (A) Group performance across time represented as a percentage of baseline performance. Significant differences between groups existed at 2-week and 6-week time points. (B) Improvement (rehab score) of each group from "post" to 2-week time point. Rehab + EGF/EPO group showed significant recovery relative to non-rehab conditions after 2 weeks of treatment (*$p<0.05$). (C) Improvement (rehab score) of each group from "post" to 6-week time point. The Rehab + aCSF group showed significantly greater recovery relative to the No Rehab + EGF/EPO group after 6 weeks of treatment (*$p<0.05$). Effects shown in B remained at 6 weeks (*$p< 0.05$).

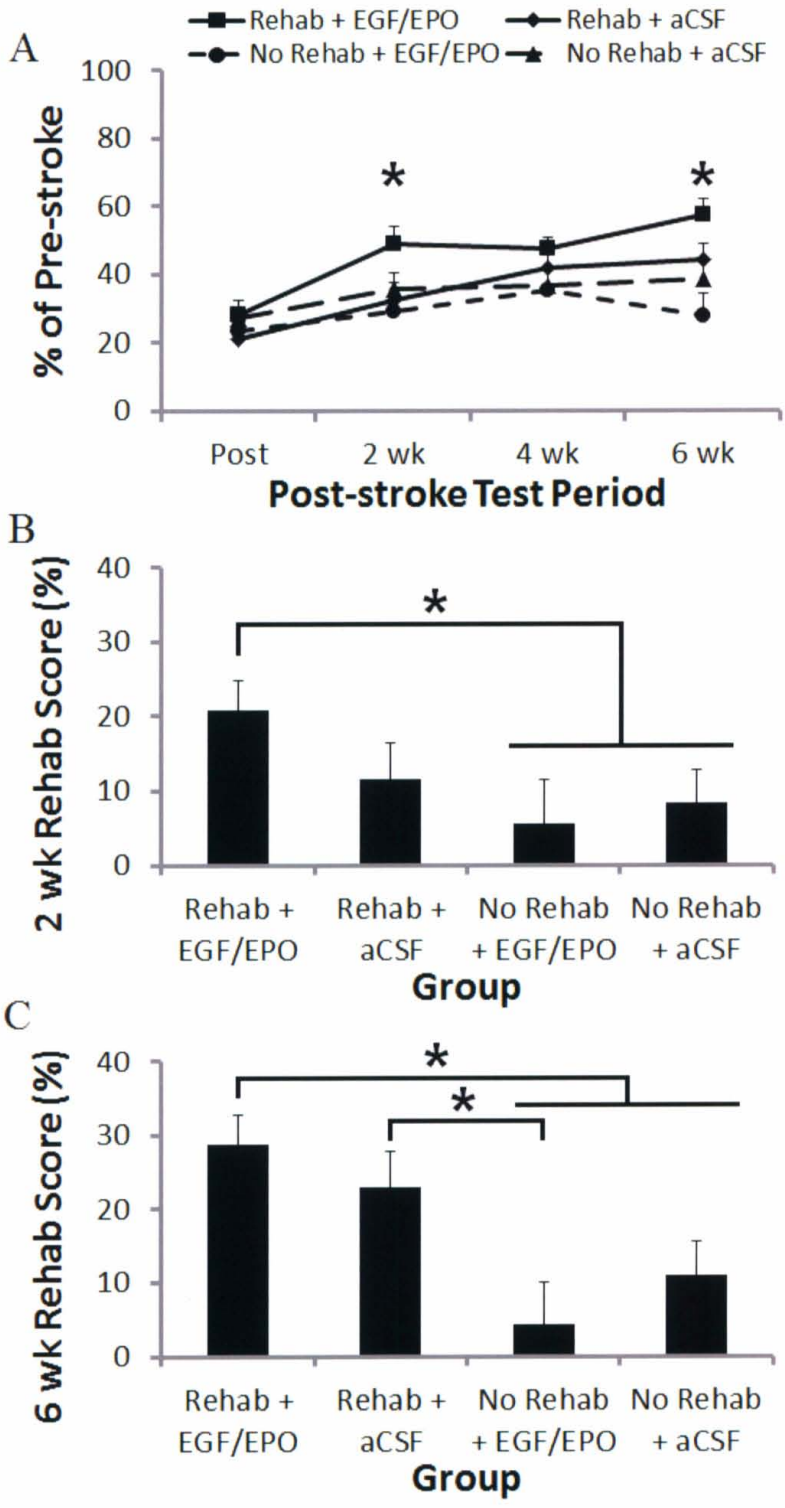
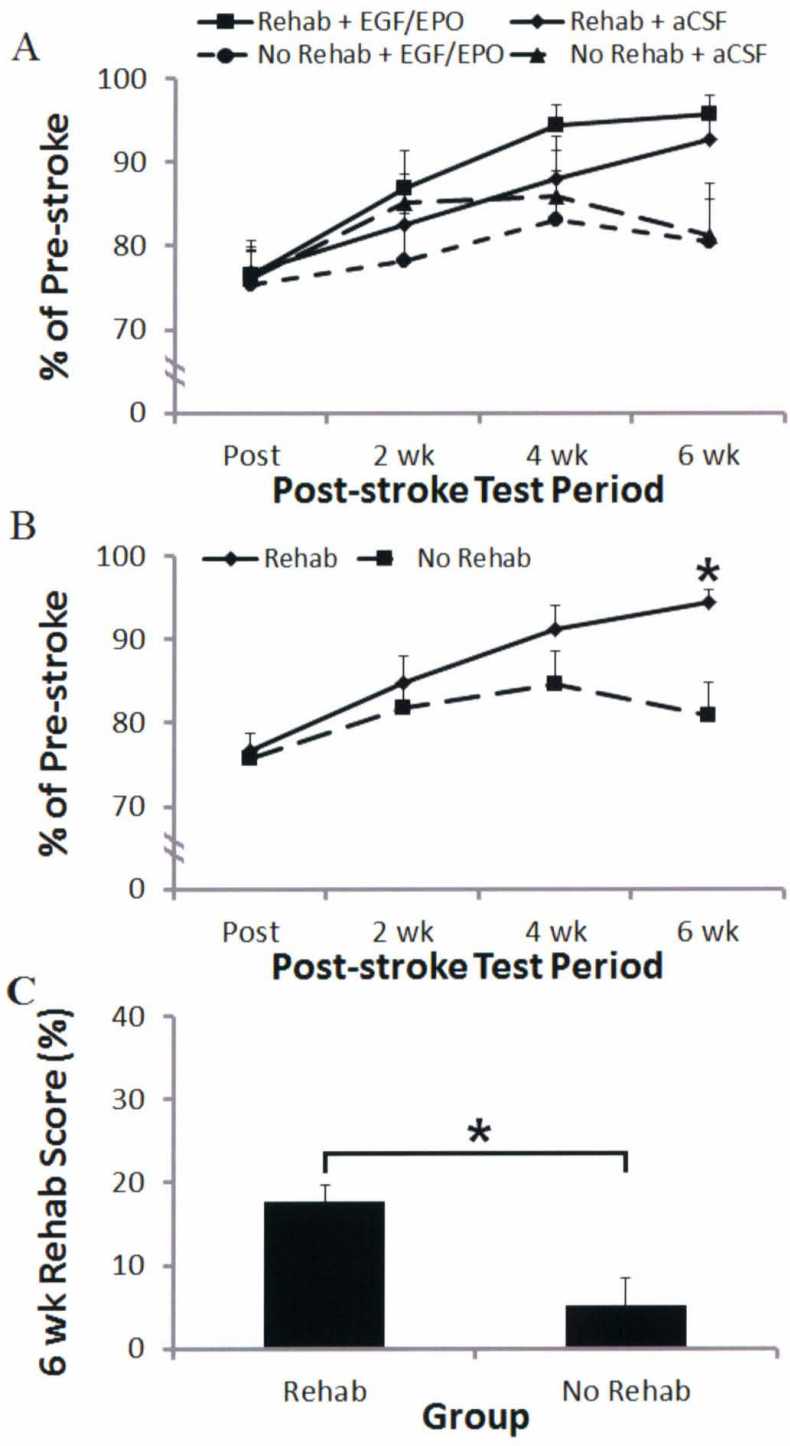

A
Rehab + EGF/EPO
Rehab + aCSF
No Rehab + EGF/EPO
No Rehab + aCSF
% of Pre-stroke
100
80
60
40
20
0
*
*
Post
2 wk
4 wk
6 wk
Post-stroke Test Period
B
2 wk Rehab Score (%)
40
30
20
10
0
*
Rehab + EGF/EPO
Rehab + aCSF
No Rehab + EGF/EPO
No Rehab + aCSF
Group
C
6 wk Rehab Score (%)
40
30
20
10
0
*
*
Rehab + EGF/EPO
Rehab + aCSF
No Rehab + EGF/EPO
No Rehab + aCSF
Group

Figure 5. Post-ischemic performance on beam-traversing task (mean ± SEM). (A) Performance of all experimental conditions across time (presented as % of baseline performance; not significant (NS)). (B) Performance of rehab conditions vs. non-rehab conditions across time. Significant differences between conditions exist after 6 weeks of treatment where animals in the Rehab condition demonstrated significant improvements over animals in the standard conditions (*$p<0.01$). (C) Improvement (rehab score) of the Rehab and No-Rehab groups from post to 6 week time point. Animals in rehab groups recovered significantly more by this time point than animals in groups that did not receive rehab (*$p<0.01$).


A
Rehab + EGF/EPO
Rehab + aCSF
No Rehab + EGF/EPO
No Rehab + aCSF
% of Pre-stroke
100
90
80
70
0
Post
2 wk
4 wk
6 wk
Post-stroke Test Period
B
Rehab
No Rehab
*
% of Pre-stroke
100
90
80
70
0
Post
2 wk
4 wk
6 wk
Post-stroke Test Period
C
6 wk Rehab Score (%)
40
30
20
10
0
*
Rehab
No Rehab
Group

Subsequent analysis on rehab score at each time point revealed that performance in the Rehab condition was significantly greater than that of the No Rehab condition after 6 weeks of treatment ($t_{48} = 10.405$, $p < 0.01$) (Figure 5C).

3.1.3 Cylinder Test of Forelimb Asymmetry

Three way-repeated measures ANOVA indicated no Time X Rehab X Pump interaction ($p > 0.05$). However, both a significant Time X Pump ($F_{2,92} = 4.198$, $p < 0.02$) and Time X Rehab ($F_{2,92} = 4.064$, $p < 0.02$) interaction was exhibited in the cylinder test (Figures 6B and C respectively). Independent samples t-tests at each test-point by Rehab condition revealed that animals in the Rehab condition increased the use of their impaired forelimb significantly more during the cylinder test than those in the No Rehab group after 2 weeks of treatment ($t_{48} = 5.359$, $p < 0.03$) (Figure 6D). Similar t-tests at each Time X Pump interaction did not reveal significant time points at which group differences existed in use of the impaired forelimb for postural support ($p > 0.05$).

3.2 Severity of Ischemic Damage

A Kruskal-Wallis non-parametric test was used to assess individual group differences on damage score ($K3 = 1.817$, $p > 0.05$). Two Mann-Whitney U tests were used to assess the main effects of Rehab ($U = 237$, $p > 0.05$) and Pump ($U = 264$, $p > 0.05$) on damage score. All of these tests failed to detect significant differences among groups, signifying that neither the pumps used, nor rehabilitation paradigm received had a significant effect on ET-1 induced maximal cortical damage (Figure 7A and B).

Figure 6. Post-ischemic performance on cylinder test of forelimb asymmetry (mean ± SEM). (A) Performance of all groups on the cylinder test of forelimb asymmetry across all test periods (NS, $p>0.05$). (B) There was a significant effect of Pump across Time. However, no differences at individual time points could be isolated. (C) There was a significant effect of Rehab across Time, but again these differences could not be isolated with post-hoc comparisons. (D) After 2 weeks of treatment, animals in the enriched rehabilitation group had recovered to a significantly greater extent than animals not receiving rehabilitation (*$p<0.05$).

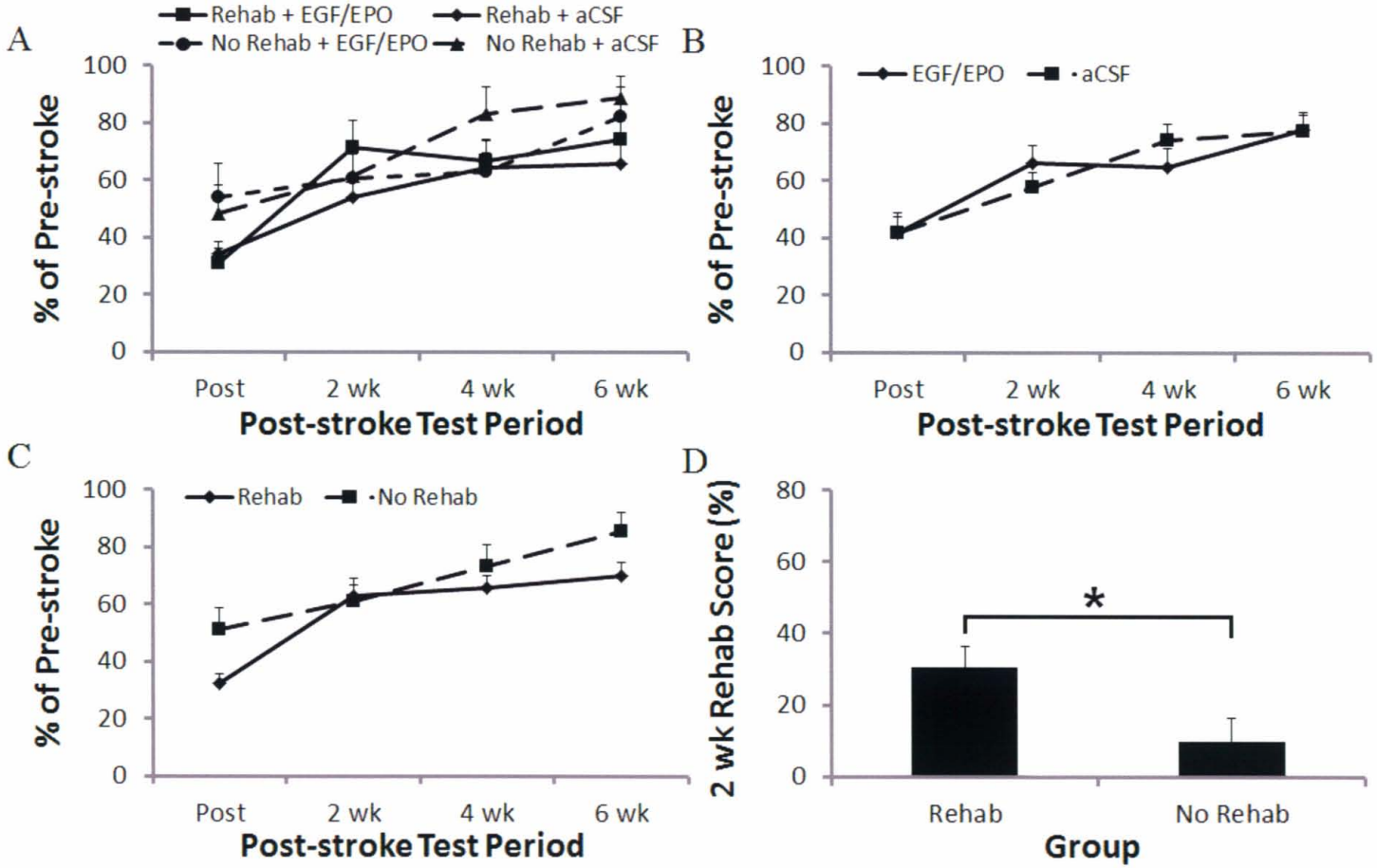
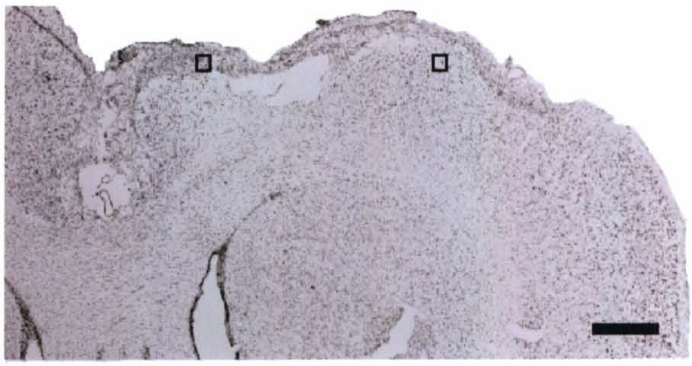
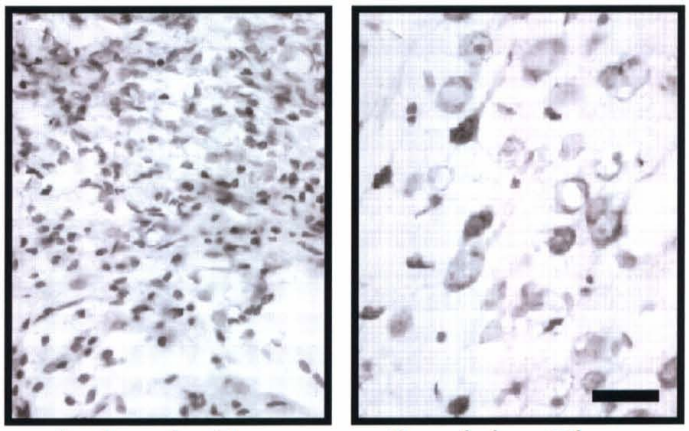

A
Rehab + EGF/EPO
Rehab + aCSF
No Rehab + EGF/EPO
No Rehab + aCSF
% of Pre-stroke
100
80
60
40
20
0
Post
2 wk
4 wk
6 wk
Post-stroke Test Period
B
EGF/EPO
aCSF
% of Pre-stroke
Post-stroke Test Period
C
Rehab
No Rehab
% of Pre-stroke
Post-stroke Test Period
D
2 wk Rehab Score (%)
*
Rehab
No Rehab
Group

Figure 7. Assessment of maximal damage to cortical tissue. (A) Representative section of damage induced by ET-1 corresponding to a damage score bordering between 2 and 3. Scale bar represents 500 μm. (B) 40X magnification of insets from ischemic (left) and surviving (right) tissue from figure A. Damaged tissue in ischemic core is condensed and more darkly stained than surviving tissue. Scale bar represents 40 μm. (C) Damage scores across groups. No differences in cortical damage were detected among groups.

A

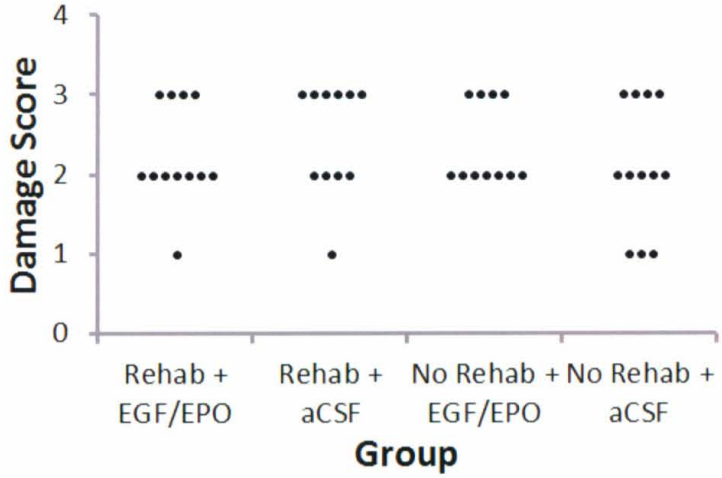


B



Ischemic Core Surviving Tissue

C



Discussion

4.1 Summary of Findings

In this study we assessed the combinatory effects of growth factor infusion (EGF/EPO) and rehabilitation in aiding recovery of motor function following ischemic stroke. We replicated previous findings that rehabilitative therapies are efficacious following motor cortex damage (Jones et al., 1999; Biernaskie et al., 2001). More importantly, however, we are the first to demonstrate that the improvements experienced from rehabilitation can be accelerated by four weeks. Administration of EGF/EPO in combination with rehabilitative therapy resulted in maximal improvements after only two weeks of treatment, whereas it required six weeks for similar improvements to be achieved with rehabilitation-alone. These data may have important implications for designing new therapies and augmenting current rehabilitation paradigms.

After just two weeks of the combination treatment of EGF/EPO and rehabilitation, animals had improved to a significantly greater extent on the staircase task than animals in the growth factor only and control groups. In contrast, the group that received rehabilitation with a vehicle infusion did not improve significantly over other groups until after six weeks of rehabilitation. Other studies of post-stroke rehabilitation have shown that significant improvements in function require two to four weeks of rehabilitation to manifest (Biernaskie et al., 2001). The daily task-specific reaching component of the present rehabilitation paradigm was intended to provide an opportunity to practice the specific movements required in the staircase reaching test. This models the reality of clinical rehabilitation, in which the goal is usually to provide restoration of motor abilities that are important in the patient's daily life. These staircase results indicated that task-

specific motor recovery was sharply accelerated by the combination of EGF/EPO and rehabilitation. However, overall level of recovery did not differ between the combination and rehab only groups in skilled reaching performance upon the conclusion of the rehabilitation program.

Interestingly, gross walking abilities, as assessed by the beam-traversing task, were not subject to the same acceleration effect as observed in the staircase reaching task. After two weeks of rehabilitation, all groups had improved their performance on the beam task to the same degree. Upon conclusion of the six week rehabilitation period, both groups receiving rehabilitation had improved significantly more on the beam walking task than animals in either of the non-rehabilitation conditions. This indicates that benefits of the combinatorial treatment may be isolated to the motor domains undergoing the most rigorous and specific therapy. It has been previously noted that recovery is enhanced for tasks that are specifically targeted by a given rehabilitation program (Richards et al., 1993).

Assessment of post-stroke asymmetrical use of forepaws for postural support using the cylinder task revealed that animals in both of the rehabilitation conditions recovered significantly more use of their impaired forelimb after two weeks of treatment than animals that did not receive rehabilitation. As with the beam task, animals in the combination treatment group recovered to a similar degree as animals in the rehabilitation only group. This is a further indication that the accelerated recovery found in skilled reaching performance may be confined to motor domains specific to the task used for rehabilitation. Upon conclusion of the rehabilitation period, all groups had recovered to

the same degree on the cylinder task, as has been noted in other studies using this test (Clarke et al., 2009).

4.2 Comparison to Previous Literature

Although this study demonstrated a beneficial effect of EGF/EPO infusions on stroke recovery when combined with rehabilitation, this differs from previous literature that has shown that EGF/EPO infusions are effective without rehabilitation. Kolb et al. (2007) showed that infusing EGF/EPO into the lateral ventricle caused significant improvements in post-stroke recovery of function over animals that received EGF, EPO, or aCSF alone. In the present study, infusing EGF/EPO alone did not result in post-stroke recovery of function that was greater than control animals on any behavioural test. There are several key differences between these studies that may explain this incongruence.

The method of stroke induction (present study, ET-1; Kolb study, pial vessel strip) differs between these studies. It is possible that different methods of injury may result in different levels of neuroblast and astrocytic migration to the site of ischemic injury. In order to administer the pial vessel strip, Kolb and colleagues removed a large portion of skull (21 mm^2) which was never replaced. Damage to the skin causes a release of many growth factors including EGF, FGF and VEGF into the local area over a period of weeks (Barrientos et al., 2008). These growth factors have been previously shown to enhance neuroplastic change as well as promote migration of new cells to the site of brain injury (Fisher et al., 1995; Zhang et al., 2000; Gregg & Weiss, 2003). The brain being in direct contact with these additional growth factors from the incised skin is potentially an important factor in promoting recovery using the pial strip method, which could lead to

an enhanced migration of beneficial cells to the peri-infarct region. With ET-1 injection, only a small hole is made in the skull (1 mm^2) meaning that the brain and skin are less able to interact, possibly resulting in less exposure to growth factors than with the pial strip model. In naturally occurring stroke, the brain neither interacts with the skin nor receives traumatic damage from permanent removal of pial vessels. For these reasons, we believe that ischemic damage resulting from ET-1 injection more closely resembles clinical stroke and these results more accurately represent the effects of EGF/EPO infusion.

Another difference between these studies is the test battery that was used to assess motor function (present study: staircase, beam, cylinder; Kolb study: tray reaching, swimming, cylinder). It is possible that had we used the same tasks as Kolb et al., we may have observed similar beneficial effects of the EGF/EPO infusion alone. Both studies measured forelimb asymmetry using the cylinder task, however, Kolb and colleagues did not observe improvements in control groups over time as was observed in the present study as well as throughout the literature (Schallert et al., 2000; Shanina et al., 2006; Clarke et al., 2009). Had this been the case, any beneficial effects of EGF/EPO infusion alone could have been masked.

In the present study, osmotic pumps were not tested upon completion of drug infusion to ensure that proper administration of drug treatment had occurred. Although all infusion pumps were prepared at similar times and inserted by an experimenter blind to the experimental conditions, it is unknown whether equal infusions of EGF and EPO were delivered to all animals. This may have led to some animals in either the combination or drug-only group not receiving the entirety of their growth factor infusion

and therefore performing more similarly to the rehabilitation-only or control groups. Exclusion of animals that had non-functioning pumps may help amplify existing differences and should be considered in future studies. This is unlikely the case however, because of the significant improvements observed in the combinatory condition. It is unlikely that only the pumps in the growth factor-alone condition were malfunctioning as these osmotic pumps have been found to have very low rates or no failure in other studies (Hewitt & Corbett, 1993; Peeling et al., 2001; Chaulk et al., 2003). A more parsimonious explanation is that administration of growth factors-alone does not provide significant recovery of function.

4.3 Possible Mechanisms for Task-Specific Accelerated Recovery

As previously mentioned, infusing EGF and EPO into the brain is thought to enhance migration and differentiation of neuroblasts into the site of ischemic damage (Gregg & Weiss, 2003; Wang et al., 2004; Kolb et al., 2007). This generates a small population of cells in the peri-infarct region that have the capacity to differentiate into new neurons and potentially replace destroyed neuronal tissue. Alternatively, these cells may assume an astrocytic phenotype and express growth factors that support cell survival and neuroplasticity in the surrounding region (Ridet et al., 1997; Chen & Swanson, 2003). Either of these possibilities could clearly provide the environment necessary to improve post-stroke motor recovery, however, our data show that animals receiving only the EGF/EPO infusion did not improve more than controls on any test of functional recovery. Additionally, animals receiving the combination treatment neither improved their overall level of performance to a greater extent nor accelerated reacquisition of motor abilities

over those receiving rehabilitation alone on tests of generalized motor ability. Accelerated motor recovery was only noted for the staircase test that is targeted by the task-specific component of the rehabilitation paradigm.

Task-specific rehabilitation may be critically important to fully utilizing the beneficial effects of exogenous growth factor infusion into the brain. It is widely thought that newly formed connections in the brain require activity in order to be maintained (Butz et al., 2009). Without receiving stabilizing growth factors or being strengthened with long-term potentiation, new synapses can be pruned (Le Be & Markram, 2006). With daily reaching rehabilitation, task-specific pathways receive intense stimulation over the course of weeks. When combined with the enhanced neuroplasticity expected from the EGF/EPO infusion, cortical maps involved in task-specific motor patterns may be able to reform and enlarge at an accelerated rate relative to motor maps involved in other stroke-disrupted movements. Evidence of this occurs naturally, when cortical representations of a limb enlarge in response to motor learning (Karni et al., 1995). This response would likely be exaggerated in an animal receiving growth factor treatment intended to enhance its inherent neuroplasticity.

Combining EGF/EPO infusion with rehabilitation accelerates the rate at which rehabilitation-mediated functional recovery occurs. However, this accelerated recovery is limited to motor domains that undergo task-specific rehabilitation. General motor abilities show improvements in recovery, but not more than is achieved with rehabilitation alone. This is speculated to be due to a domination of neuroplastic resources by the motor pathways undergoing the intense stimulation of task-specific

rehabilitation, but further study is required in order to elucidate the true mechanisms behind this accelerated motor recovery effect.

4.4 Limitations

There is an aspect of the present study that limits the extent to which interpretations of the results can be made. The window in which animals were tested restricts the interpretation of the rate and absolute level of functional recovery. Because animals in the combination group had already reached their maximal level of recovery by the first testing point after the onset of rehabilitation, it is impossible to determine the timeline of recovery. Animals in the combination group may have reached maximal motor recovery on the staircase test anywhere from 0-14 days following the commencement of the rehabilitation paradigm. Furthermore, animals receiving the combination treatment still appeared to be improving their performance after six weeks of treatment, indicating that continuing rehabilitation and testing may have further improved the performance of these animals. Despite this limitation to determining the absolute effects of the combination treatment, the finding that performance on a test of skilled reaching ability is significantly improved after two weeks of rehabilitation still stands. This study demonstrates that combining growth factors with rehabilitation in rats following ET-1 ischemia significantly reduces treatment times for maximal recovery. Addressing this limitation in subsequent studies could show that this combination therapy is even more beneficial than initially anticipated.

4.5 Conclusion

The effect of combining growth factor infusion with rehabilitation for post-stroke recovery of function is a promising treatment, especially for severe stroke where rehabilitation provides limited benefit (Carey et al., 1988; Asberg & Nydevik, 1991; Alexander, 1994). By accelerating the rate at which lost motor abilities are recovered after stroke, the overall burden of stroke on society can be substantially diminished in many ways. Reduced treatment time could result in a drastic reduction of direct health care costs for treating each stroke patient. With fewer resources required to treat each patient, medical personnel would be available to treat more individuals, which will be increasingly important in Western, aging societies. Perhaps most importantly, enabling 'normalcy' in individuals who have suffered a stroke as quickly as possible is crucial for not only the economic health of our society, but also for ensuring that every person can attain the highest quality of life.

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