Published in final edited form as:
Neurobiol Dis. 2010 February ; 37(2): 237. doi:10.1016/j.nbd.2009.09.005.

Translating the Frontiers of Brain Repair to Treatments: Starting Not to Break the Rules

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Abstract

The field of neural repair in stroke has identified cellular systems of reorganization and possible molecular mechanisms. Conceptual barriers now limit the generation of clinically useful agents. First, it is not clear what the causal mechanisms of neural repair are in stroke. Second, adequate delivery systems for neural repair drugs need to be determined for candidate molecules. Third, ad hoc applications of existing pharmacological agents that enhance attention, mood or arousal to stroke have failed. New approaches that specifically harness the molecular systems of learning and memory provide a new avenue for stroke repair drugs. Fourth, combinatorial treatments for neural repair need to be considered for clinical therapies. Finally, neural repair therapies have as a goal altering brain connections, cognitive maps and active neural networks. These actions may trigger a unique set of "neural repair side effects" that need to be considered in planning clinical trials.

Keywords

Stroke; axonal sprouting; stem cell; brain map; cortex; regeneration; translational; clinical trial

> "If a rule prevents you from improving or maintaining Wikipedia, ignore it."
> http://en.wikipedia.org/wiki/Wikipedia:Ignore_all_rules

Neural repair started as a field that ignored the rules. The rules consisted of certified CNS dogma about the static nature of brain structure and connectivity. Examples include the rule that the adult brain formed new connections only in certain specialized and highly plastic structures, such as the hippocampus; or, that regions of the adult brain did not develop new populations of neurons. Early studies in neural repair after stroke suggested that growth-associated proteins commonly linked to axonal growth cones were induced in humans and animals in peri-infarct tissue (Ng et al., 1988; Stroemer et al., 1995). Later studies extended these findings with quantitative analysis of axonal connections to show that the adult brain forms new connections in peri-infarct cortex, and in projections from cortex opposite to the stroke (Carmichael et al., 2001; Chen et al., 2002; Dancuase et al., 2005). Later studies in neural repair suggested a fantastic biology—that not only was the "no new neuron" dogma wrong, but stroke signaled for a long distance migration of newly born neurons through several different CNS tissue compartments to regions of damage after injury (Arvidsson et al., 2002; Parent et al., 2002; Zhang et al., 2004; Ohab et al., 2006). On closer examination of the literature, these "rules" on static CNS structure were actually more commonly accepted beliefs.

Evidence has been present for some time that synaptogenesis or cortical growth occurs in the adult as a result of activity and injury (Eccles, 1976; Kolb et al., 1983; Greenough et al., 1985). Neurogenesis in the olfactory and hippocampal systems in normal and injured states was well described for some time before its expansion was recognized in stroke (Altman and Das, 1965; Reznikov, 1975; Kaplan and Hinds, 1977). The key evolution in this field is the recognition that these structural changes may underlie at least a component of functional recovery, and may interact with biochemical and electrophysiological changes in the brain after stroke to form CNS systems that can be manipulated to promote brain repair. A next key step in the field of neural repair after stroke will now be to bring the new concepts of tissue reorganization and recovery back under the rules of translational medicine so as to move these new therapeutic ideas into the clinic. This process of translating the frontiers of brain repair requires a focused examination of these frontiers and how they might be used for treatments.

What is neural repair?

All stroke patients exhibit some degree of functional recovery. This process occurs in a matter of days on the acute stroke service, and continues most dramatically for the first month in upper and lower extremity motor function (Kreisel et al., 2007) and for up to a year in language and other cognitive modalities (Hier et al., 1983; Kauhanen et al., 2000). This recovery is not complete, leading to the tremendous long term personal and financial burdens of this disease (Carmichael, 2006; Benowitz and Carmichael, 2010). What mediates neural repair in stroke and what are the pharmacological targets to promote improved recovery? There is clear, if somewhat anecdotal, evidence for a regression of acute stroke damage as a mechanism of early recovery. This process includes a reduction in cerebral edema and a waning in the initial phases of inflammation (Dobkin, ‘03). Other mechanisms of neural repair in stroke are associated with anatomical and physiological plasticity induced by the infarct: axonal sprouting, neurogenesis, angiogenesis, electrophysiological measures of neuroplasticity and reshaping of distributed cortical networks. These processes have been recently reviewed (Floel and Cohen, 2010; Wittenberg, 2010; Benowitz and Carmichael, 2010).

Many of these processes of structural and physiological change after stroke have been correlated with recovery but the causal mechanisms of neural repair in stroke have not been defined. Axonal sprouting from the cortex contralateral to an infarct into the cervical spinal cord and brainstem ipsilateral to the infarct correlates with recovery of forelimb use (Chen et al., 2002; Papadopoulos et al., 2002). Neurogenesis after stroke is associated with functional recovery, in that blocking mitotic activity after stroke reduces cognitive recovery (Raber et al., 2004). The degree of angiogenesis after stroke in humans is correlated with the level of recovery (Krupinski et al., 1993). Stem cell, growth factor and cytokine therapies that promote functional recovery correlate with increases in angiogenesis and neurogenesis near the infarct (Zhang and Chopp, 2009; Bliss et al., 2010).

However, a key issue in the translation of an experimental neural repair treatment to a clinical therapy will be to identify which of these biological events causally supports recovery. In other words, a rule in clinical translation is to know what cellular process a drug/molecule is affecting that mediates its clinical efficacy. If this is not known, side effects cannot be predicted or understood, biomarkers of repair cannot be developed with any real link to the actual repair process (i.e. they will not really “mark”), and clinical subsets of patients with varying treatment responses will not be interpretable. As an example, if a cytokine, growth factor or inhibitory protein blocker promotes axonal sprouting from contralateral cortex to spinal cord, is this the pathway that mediates recovery? Or are other pathways also sprouting, and these, though possibly not examined in a published study, mediate recovery? What happens if a patient exhibits a dystonic movement posture because of sprouting in corticostriatal pathways after a neural repair drug in stroke? Should this be tolerated because this is the pathway that mediated

their improved motor function, or did the improved motor function come about from corticospinal sprouting and this dystonia is an untoward effect? Recent studies in the field of stem cell transplantation field have begun to identify causative mechanisms in the neural repair and recovery after transplantation (Bliss et al., 2010). Future studies will need to identify the causal mechanisms of normal neural repair and of drug-induced neural repair so as to follow the clinical translational rules.

Delivery Issues: translating identified molecular systems into human therapies

Several molecular systems appear to induce or block neural repair after stroke and other CNS injuries. The serine/threonine kinase Mst3b is induced by inosine and mediates axonal sprouting and behavioral recovery (Chen et al., 2002; Zai et al., 2009). NogoA blocks axonal sprouting, and interfering with Nogo function produces behavioral recovery after stroke (Papadopoulos et al., 2002; Lee et al., 2004; Zai et al., 2009). EPO and G-CSF interact with mechanisms of early cell death and neural repair and mediate behavioral recovery after stroke (Maurer et al., 2008). The protein phosphatase PTEN, which inactivates inositol triphosphate signaling, dramatically controls axonal sprouting after injury in the adult CNS (Park et al., 2008). These studies represent a clear advance in the field, in that they point the way to molecular systems that can be manipulated in novel neural repair therapies. The next step in the process of clinical translation is to identify approaches that will manipulate these systems locally in the brain after stroke.

A major issue in the manipulation of an identified neural repair molecular pathway is drug delivery. Systemic drug delivery has problems of selective brain access and off-target effects. Cytokines or growth factors that stimulate neural repair and recovery, such as EPO and G-CSF, selectively permeate the blood brain barrier but carry the risk that they will have effects on bone marrow, kidney and other systemic organs. EPO has recently been the target of clinical warnings for its use in cancer because of increased cardiovascular events and death (Fishbane and Nissenson, 2007) and a clinical trial with EPO in stroke was recently suspended. Systemic administration of beta fibroblast growth factor produced significant benefits in acute stroke and neural repair after stroke in pre-clinical models (Ay et al., 1999), but caused side effects when administered systemically to humans (Clark et al., 2000). There are promising developments in this area. Modified EPO derivatives that target the common beta cytokine receptor (Siren et al., 2009) may provide a selective targeting of neural EPO activity. G-CSF stimulates the immune system after stroke, but this effect did not produce side effects in a small clinical series (Sprigg et al. 2006). As G-CSF was developed to specifically boost white blood cell production, a full consideration of immunologically related side effects will await the ongoing clinical trial results.

Other growth factor candidates for neural repair in stroke do not pass the blood brain barrier. A prominent example is BDNF, which binds trkB to stimulate axonal and dendritic sprouting and promote neurogenesis (Binder and Scharfman, 2004). BDNF is an attractive candidate for a neural repair molecular therapy when delivered through invasive cannulation, but does not significantly pass the blood brain barrier if delivered systemically (Zhang and Pardridge, 2001). Prolonged intracerebroventricular cannulation for neural repair drug delivery in stroke is less than optimal because of the risk of infection and because of the need to place many stroke patients on anti-coagulation or enhanced anti-platelet therapies with bleeding side effects. Many of the initial targeting approaches to block axonal growth inhibitors utilize monoclonal antibodies or large peptides (Walmsley and Mir, 2007). These also do not cross the blood brain barrier. In stroke the blood brain barrier is opened for variable periods in the subacute phase. In experimental animals this can be precisely determined with specific molecular weight indicators (Friedman et al., 2009). Systemic administration of tagged small

molecules or antibodies shows that these will penetrate the peri-infarct cortex and modify neurogenesis and recovery up to a week after stroke in mice (Ohab et al., 2006). However, blood brain barrier function varies considerably across strains of the same experimental animal and between rodents and humans (Hermann, 2008). Data on the duration of BBB opening in stroke in humans varies by technique, to suggest either an opening near the infarct (Bang et al., 2007), an extended region of BBB opening in the ipsilateral hemisphere to stroke, or no opening despite similar stroke types (Merten et al., 1999; Lorberboym et al., 2003). An important point from this data is that neural repair drugs will need to follow an important rule of clinical translation: verify that that the drug is actually getting into the target region. Simply systemically administering a monoclonal antibody blocker to an axonal growth inhibitor after stroke, without first validating that this penetrates into the brain in humans or primates, is likely to add yet another failed trial to the pile of such failures in stroke.

An attractive drug delivery strategy in stroke is to use the stroke cavity itself as a depot site. The stroke cavity may be an ideal target. It is a cavity, because the necrotic tissue is absorbed, and can accept a large volume injection for the CNS. It is located directly adjacent to a major target site of neural repair and recovery in stroke, the peri-infarct tissue. New bioengineering approaches provide hydrogels or other delivery systems that are compatible with the brain and support drug delivery or stem cell engraftment from the stroke cavity. Indeed, local release of an anti-Nogo peptide through such an approach improves behavioral recovery without the need for prolonged intraventricular catheterization (Ma et al., 2007). Hydrogel support of transplanted stem cells allows their survival within the infarcted cavity, without an approach of multiple injections into peri-infarct tissue that may damage normal brain (Bible et al., 2009). Direct operative stereotaxic access to the stroke cavity is or will soon be commonplace in neurosurgical operating suites (Miller et al., 2008; Carmichael et al., 2008).

Learning and Memory, Peri-Infarct Cortex and Pharmacological Repair Therapies

Neurorehabilitation employs learning rules to guide therapy, such as learned non-use, mass action, contextual interference and distributed practice (Dobkin, 2003; Krakauer, 2006). These therapies for brain injury induce a reorganization in brain mapping that closely parallels that seen with memory and learning paradigms: an initially diffuse network of brain areas is gradually funneled down with training into a core set of areas directly involved in the tasks (Kelly et al., 2006; Butefisch et al., 2006). In addition to brain imaging correlates, the processes of learning and memory and neuroplasticity after stroke share similar molecular mechanisms. Genes that are important in learning and memory also are upregulated during neural repair after stroke, including the stathmin family genes SCLIP and SCG-10 (Peng et al., 2003), the membrane-associated phosphoproteins GAP43 and MARCKS (Holahan and Routtenberg, 2008; Solomonia et al., 2008), the transcription factor c-jun (Tischmeyer et al., 1994) and the cell adhesion molecule L1 (Arami et al., 1996) (Carmichael et al., 2005). On a cellular level, similar types of neuronal responses are seen in stroke and in learning and memory in terms of dendritic remodeling (Brown et al., 2007) and LTP (Hagemann et al., 1998). With theses similarities in cognitive training principles, brain imaging, effector proteins and cellular responses, it has long been supposed that the molecular correlates of learning and memory are the underpinnings of recovery or compensation from brain injury.

This idea that some aspect of learning is the neuronal basis for recovery has led to ad hoc attempts to treat brain injured patients with any available drug that might also stimulate learning, memory or attention: including serotonin reuptake inhibitors, dopamine agonists, methylphenidate, modafinil and amphetamine. Because these drugs were developed to treat conditions other than neurorehabilitation from brain injury, and because they act with less specificity on many neurotransmitter systems in the brain, their role in promoting neural

recovery after brain injury has not withstood rigorous clinical trials (Platz et al., 2005; Sprigg et al., 2007; Cramer et al., 2009). However, studies of molecular memory systems suggest several targets for a pharmacological learning therapy in stroke. Several classes of drugs, known loosely as "cognitive enhancers", may directly stimulate learning and memory and may be useful in stroke: AMPAKines (positive allosteric modulators of the AMPA receptor), extrasynaptic GABAα receptor antagonists, nictonic receptor subunit alpha 7 acetylcholine receptor agonists and phosphodieseterase 4 inhibitors (Barad et al., 1998; Lynch, 2006; Bitner et al., 2007; Atack, 2008). PDE4 inhibitors do modulate motor map plasticity and recovery in stroke (MacDonald et al., 2007). The other drug classes have not yet been tested in stroke.

Memory formation passes through specific molecular steps. Postsynaptic memory induction involves αCamKII. αCAMKII is a major component of the dendritic spine, involved in spine turnover, mediates LTP and plays a critical role in the induction of memory (Okamata et al., 2006; Yamauchi, 2005). NMDA-dependent autophosphorylation of αCAMKII at threonine-286 is a key initial step for the induction of synaptic plasticity (e.g. LTP) and learning. Pre-synaptic memory induction is dependent on the activation of the small GTPase Ras. Ras activation triggers a phosphorylation cascade through sequential steps: Ras/Raf/Mek/MAPK (Pearson et al., 2001). In the bouton, one mechanism for the modulation of LTP induction in this system is phosphorylation of synapsin, which facilitates synaptic glutamate release (Kuchner et al., 2005). Memory consolidation involves a cascade that converges on the transcription factor CREB. Genetic knockdown of CREB function in pyramidal neurons results in deficits in CREB gene induction and maintenance of memories (Kida et al., 2001), whereas viral gene delivery of CREB produces enhanced expression of immediate early genes following memory testing, a greater chance of these neurons being incorporated into memory traces, and facilitated memory (Han et al., 2007). Can drugs that stimulate these learning and memory systems promote neural repair and recovery in stroke? The answer to this question does not so much utilize a clinical translational rule as take from a valued pharmacological industry practice: it is far easier to test a library of drugs developed for a molecular mechanism for one biological process (memory) and apply it to another (stroke) than it is to develop a disease-specific class of drugs de novo.

Targeting learning and memory systems to promote neural repair after stroke may also have its dark side. One intriguing possibility is that activating an enhanced plasticity state in the adult after stroke may interfere with that most plastic of brain activities, learning and memory. An example of this is in the recently published effects of one major neural repair strategy, digestion of glial inhibitory molecules. Chondroitin sulfate proteoglycans (CSPGs) are extracellular glial axonal growth inhibitors that are an emerging target for neural repair in stroke. Delivery of the degradative enzyme chondroitinase ABC produces widespread removal of an active moiety in CSPGs even with single injections, and produces axonal sprouting and recovery in CNS lesions. CSPGs form the perineuronal net around neurons in the adult (Galtrey and Fawcett, 2007). Forming the peri-neuronal net is a process that leads to the mature adult brain state (Pizzorusso et al., 2002), and perineuronal nets are important for stable representation of long-term memories (Gogolla et al., 2009). It is possible that a neural repair therapy such as chondroitinase may not only allow, say, axonal sprouting in motor circuits and improved arm control after stroke, but have an "on-target effect" like disruption of stable encoding or retrieval of memories.

Combinatorial Treatments

The active promotion of neural repair after stroke may necessitate combination therapies. Data from initial spinal cord injury studies and axonal sprouting studies in optic nerve and stroke injury models indicates that it is not enough to simply block an axonal growth inhibitory system, such as the NogoA system (Benowitz and Carmichael, 2010). Adult neurons fail to regenerate

appreciably, even with a more permissive environment, because they are not in a “growth state”. The Benowitz lab has been a pioneer in this concept of delivering combinatorial treatments that both block axonal sprouting and induce a neuronal growth state. Inosine activates the kinase Mst3b and, in combination with a Nogo antagonist, markedly enhances axonal sprouting in the denervated cervical spinal cord (Zai et al., 2009). This activation of a neuronal growth state may resemble in some aspects placing an adult neuron into a more embryonic growth condition (Benowitz and Carmichael, 2010). It is possible that behavioral activity paradigms may substitute for the “growth induction” drug. Increased behavioral activity either generally, as in environmental enrichment, or specifically, as in forced use of the affected limb, have a dose response, timing and brain region-specific effect on neuronal plasticity and behavioral recovery in stroke and CNS lesions (Farell et al., 2005; Conner et al., 2005; Kleim and Jones, 2008). These behaviors specifically activate motor circuits (Ferezou et al., 2007) that are affected in stroke. It is likely that the effect of the behaviors is mediated in part by local release of growth factors such as BDNF (Neeper et al., 1996) in the active and reorganizing areas with skilled reach and the running and other stimuli of environmental enrichment.

This interaction of activity and recovery in stroke pre-clinical studies has three possibilities for the application of rules of translational medicine. First, most candidate stroke patients will be undergoing activity based behavioral patterns on a neurorehabilitation unit. To fully determine the effect of a neural repair therapy, pre-clinical studies will need to incorporate some representational model of human neurorehabilitation. Second, both rodents and patients experience true recovery and also compensation over time in stroke (Levin et al., ’09). It is likely that these two processes have their own kinetics, neuronal circuits and responses to activity and to neural repair therapies. The ultimate outcome measure is functional accomplishment of a previously impaired activity. But it is important in both pre-clinical and clinical studies to determine whether recovery or compensation is mediating this ultimate outcome. Decisions on types of drugs, dosing and activity paradigms may change on this basis. Third, laboratory rodents are socially isolated, environmentally deprived and physically inactive for generations. Exposure to environmental enrichment or skilled limb training likely activates a burst of molecular and cellular neuroplasticity in a deprived brain that is primed for it. However, human stroke occurs in free-range, exploratory, stimulated people. Unlike lab rats, people with stroke may have a high degree of activation of endogenous brain plasticity systems, and not experience the dramatic boost in neural repair that is seen in rats with activity-based therapies.

Conclusion: What is a tolerable side effect profile in repairing the brain?

Assuming that specific CNS drug or stem cell delivery issues have been worked out, neural repair therapies in stroke will, by definition, activate brain plasticity in the context of injury and reorganization. For a neurologist, ischemic stroke is characterized almost entirely by negative clinical symptoms: loss of function in the damaged brain circuits. Compared with other forms of brain injury, there are few “positive” symptoms in stroke. For example, less than 10% of ischemic stroke patients will develop epilepsy in 5 years (De Reuck, 2007). Very few patients develop positive motor symptoms such as dystonia or tremor and few patients develop what might be termed positive cognitive symptoms, such as hyperactivity, mania or emotional lability (except for large or recurrent strokes producing pseudobulbar palsy). An effective neural repair therapy, whether it is a drug or stem cell treatment, is designed to activate axonal sprouting, local growth factor production or enhance neuroplasticity in injured or adjacent cortical circuits. It may be entirely possible that enhancing plasticity in these circuits improves, say, motor control of an affected limb or of language expression. But it is also possible that enhanced plasticity in the injured state, within either the target brain systems or other CNS areas, increases the chance of post-stroke epilepsy, or a movement disorder such

as tremor or dystonia. Such a possibility has occurred in pre-clinical studies in spinal cord injury, where activation of axonal sprouting has induced a neuropathic pain syndrome (Deumens et al., 2008). Difficult to treat dystonias occurred after fetal cell transplantation in Parkinson's Disease (Freed et al., 2001).

The key rule of translational medicine is to do no harm. Neural repair therapies may produce both good and harm in ways that require very active consideration. What happens if a patient has medically controllable seizures, but can talk effectively because of a neural repair drug? What if a patient has minor but daily and uncontrolled changes in shoulder posture (dystonias), but can use that arm in her activities of daily living because of a stem cell therapy after stroke? These are considerations that need to be made when the goal is to unlock the normal adult CNS plasticity blockade in the formation of new connections, the widespread alteration of cognitive maps, and in the modification of usually conservative brain changes in response to practice and learning. In this case it may be considered that a clinical translational rule could be ignored.

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