Published in final edited form as:
Dev Neurobiol. 2007 August ; 67(9): 1133–1147. doi:10.1002/dneu.20514.

Curiosity and Cure: Translational Research Strategies for Neural Repair-Mediated Rehabilitation

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Abstract

Clinicians who seek interventions for neural repair in patients with paralysis and other impairments may extrapolate the results of cell culture and rodent experiments into the framework of a preclinical study. These experiments, however, must be interpreted within the context of the model and the highly constrained hypothesis and manipulation being tested. Rodent models of repair for stroke and spinal cord injury offer examples of potential pitfalls in the interpretation of results from developmental gene activation, transgenic mice, endogeneous neurogenesis, cellular transplantation, axon regeneration and remyelination, dendritic proliferation, activity-dependent adaptations, skills learning, and behavioral testing. Preclinical experiments that inform the design of human trials ideally include a lesion of etiology, volume and location that reflects the human disease; examine changes induced by injury and by repair procedures both near and remote from the lesion; distinguish between reactive molecular and histologic changes versus changes critical to repair cascades; employ explicit training paradigms for the reacquisition of testable skills; correlate morphologic and physiologic measures of repair with behavioral measures of task reacquisition; reproduce key results in more than one laboratory, in different strains or species of rodent, and in a larger mammal; and generalize the results across several disease models, such as axonal regeneration in a stroke and spinal cord injury platform. Collaborations between basic and clinical scientists in the development of translational animal models of injury and repair can propel experiments for ethical bench-to-bedside therapies to augment the rehabilitation of disabled patients.

Keywords

rehabilitation; stroke; spinal cord injury; neural repair; animal models

INTRODUCTION

Preclinical studies that reveal positive results for an experimental intervention for neuroprotection or neural repair can have an enormous impact on the expectations of clinicians, patients, and the media. The demand for cures is great. In the United States alone, sudden profound debilitation occurs every year in 400,000 people after stroke who join 3

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million chronically disabled survivors; in 50,000–100,000 with traumatic brain injury (TBI) and many more chronically disabled survivors; in 10,000 with acute traumatic spinal cord injury (SCI) and 300,000 chronically paraplegic and tetraplegic people, along with others who suffer progressive irreversible myelopathies and cauda equina lesions from degenerative spine disease; in 200,000 with chronic multiple sclerosis with demyelination and axonal lesions; and tens of thousands with other central and peripheral nerve diseases or traumatic injuries (Dobkin, 2003).

Neurobiologists are curious about neural mechanisms in states of health and disease. Clinicians aim to use that science to mollify disease. The applicability of basic research to the complex milieu of human disease has been fruitful, hopeful, and sometimes misleading. Scientists who investigate strategies for neural repair examine fundamental processes that include manipulations of the promoters, inhibitors and modulators of endogenous neurogenesis, exogenous cell transplants, axonal transport, and guidance systems needed for regeneration, cell–cell signaling in myelinated fibers, re-expression of developmental genes, dendritic and spine sprouting, assembly of synapses, and activity-dependent synaptic reorganization (Benowitz, 2007; Tuszynski, 2007; Wang et al., 2007). Research laboratories may use cell cultures to test responses of neurons or neurites to a molecule placed into the medium or employ rodent models of ischemic, inflammatory, degenerative, traumatic, metabolic, and genetic diseases to study singular mechanisms of injury and repair. These highly focused mechanistic studies, by their purposefully controlled experimental designs, do not necessarily serve as steps for the development of clinical therapies, but can suggest bench-to-bedside applications. Planned preclinical investigations set a tedious scientific and regulatory course toward establishing the conceptual basis, methods, safety, and potential for efficacy of protective and restorative interventions in patients. Media reports of successful experiments in brain or spinal cord injured rodents, however, project the hope for imminent cures of paralysis, sensory loss, bladder dysfunction, neurogenic pain, and cognitive impairments.

Neuroscientific studies about activity-dependent learning and plasticity, along with functional neuroimaging studies of training-induced cortical reorganization in animal models and patients, have injected insight and enthusiasm for new approaches for neurologic rehabilitation (Dobkin, 2004a, b; Strangman et al., 2005). Clinical researchers aim to move biologic manipulations that may lessen physical and cognitive impairments and associated disabilities in walking, use of an affected hand, and performance of daily activities into clinical trials. Studies in animal models have already led to several safety studies of human interventions for stroke. These include intravenous injection of autologous mesenchymal stem cells about 40 days after a hemispheric stroke (Bang et al., 2005) and implantation of human neuronal cells (LBS-Neurons; Layton Bioscience) into the edge of deep infarcts near the basal ganglia (Kondziolka et al., 2005). Safety studies in SCI have proceeded with injection of human fetal spinal cord tissue into the syrinx of five subjects without any negative impact or gains (Anderson et al., 2005). Trials of autologous-activated macrophage injections into the acutely injured milieu (Proneuron Biotechnologies, Israel) and porcine oligodendrocyte progenitors (www.diacrin.com) were prematurely halted, but no data has been published. Autologous olfactory ensheathing glia were transplanted into a few patients with chronic injuries without complications (Feron et al., 2005) and appeared to improve

motor scores modestly in seven subjects (Lima et al., 2006). Locally induced inflammation followed by infusion of autologous, bone marrow derived transdifferentiated neural stem cells reportedly produced modest sensorimotor gains in two SCI subjects (Moviglia et al., 2006). Dose escalation safety trials of dural/intrathecal infusions of Rho (Cethrin) and a Nogo inhibitor are in progress. No serious complications have been described to date, but little information has been published.

The offer of hope is powerful. At conferences and on Web pages, but not in peer-reviewed published reports, clinics in China (www.stemcellschina.com), Europe, Russia, off shore of the United States, and South America offer fetal, olfactory ensheathing glia, stromal, and other unspecified cell injections into the cord, brain, and cerebrospinal fluid (Enserink, 2006), as well as peripheral nerve bridging experiments for SCI. These unpublished interventions are based upon a broad interpretation of preclinical experiments in rodents. For example, over 500 patients with SCI are reported by one Chinese neurosurgeon to have received olfactory ensheathing cells injected into the cystic cavity of patients (Dobkin et al., 2006b). Claims of slight gains by patients have been made within a day after treatment, which would not be consistent with an effect on axon regeneration or remyelination (Ibrahim et al., 2006), but may be related to the transient decrease in clonus and hypertonicity described by patients, especially by those who developed fever, headache, and a cerebrospinal fluid pleocytosis postoperatively. The only published characterization of the transplants, which are cultured from two aborted fetuses per patient, suggested that the cells may not always be ensheathing glia (Guest et al., 2006). Patients with amyotrophic lateral sclerosis have traveled to this neurosurgeon to get the same cells placed into their frontal lobes via burr holes. Nothing of medical or scientific value, however, has been learned from these human experiments, because no subject inclusion and exclusion criteria exist (e.g., some spinal injured patients had incomplete lesions and could walk, others had a myelitis without a cystic lesion to target, patients are eligible months to decades after SCI); no anticipated effects of the intervention are prespecified; no standardized measures are performed before or at intervals after the surgical intervention to assess for possible complications or effectiveness of the implant procedure (five of seven implanted patients who were prospectively followed by Western clinicians developed meningitis postoperatively); no unbiased assessors test subjects; and patients and families are left to draw their own conclusions about whether any change they notice at any time after the procedure is related to the surgery (Dobkin et al., 2006b). The surgeon has spoken against any plan to test his approach by a randomized controlled clinical trial. Transplantation and other invasive biologic interventions that proceed without an unbiased design cannot inform clinicians or patients about possible risks and benefits with due diligence, so the experiments do not represent an ethical medical practice. The neurosurgeon in China, however, professes that his fetal cell injections are a logical bench-to-bedside translation that offers hope to patients. In doing so, he and other clinicians around the world misinterpret the applicability of preclinical data from animal models, exploit under-informed subjects, and undermine acceptable Western bench-to-bedside scientific methods that aim to determine if an experimental therapy is better than standard care.

Some of the potentially confounding factors that challenge investigators who try to move from preclinical experiments to clinical trials can be managed when basic and clinical

scientists work together on a translational strategy. This review examines some of the salient problems and possible solutions for the translation of bench-to-bedside research for neural repair after stroke and SCI.

Animal Models

Although not a formal requirement for evidence of efficacy, the U.S. Food and Drug Administration does require the safety of a drug at a range of dosages to be demonstrated in animal models. It would be most unusual for a clinical trial to proceed without some evidence of a physiologic or behavioral improvement for an experimental intervention compared to a placebo. Indeed, it seems most ethical to carry out rigorous preclinical experiments with an animal model designed to be relevant to the human disease, use valid and reliable histologic, physiologic, and behavioral outcome measures that have parallel outcomes in patients, and then interpret the results in keeping with its magnitude and the applicability of the response to patients. Animal models of an intervention may lead to a sequence of clinical trial phases (Table 1). The number of failed Phase 2 and 3 human trials of acute interventions for stroke, TBI, and SCI have, however, left many scientists and clinicians wondering whether translational research based on rodent models is feasible. Despite seemingly positive results in rodent models of injury in which many types of intervention have reduced the volume of the lesion or improved a behavior, experimental pharmacologic interventions have failed to improve patient outcomes in at least 125 randomized clinical trials in stroke and perhaps half that total for TBI and SCI. These models of ischemia and trauma were simulations of human disease in which the investigators, and especially the pharmaceutical maker, emphasized a mechanism of action and treatment effect that were probably too modest to have an impact on profound stroke, TBI or SCI in patients. Only one or two acute interventions for each disease have shown some benefit, and even those results are controversial (Bracken et al., 1998; Narayan et al., 2002; Albers et al., 2004; Lees et al., 2006). Clinical investigators continue to try to understand and correct the failure to translate rodent studies into successful clinical trials (Roundtable, 1999). These errors of omission and commission in translational trials of neuroprotection hold lessons for neural repair trialists. Other practical lessons can be learned from the incremental progress earned through reiterative rodent and nonhuman primate experiments, setbacks, and problem-solving strategies that led to some success in human trials of cell transplantation for Parkinson and Alzheimer disease (Piccini et al., 2005; Tuszynski et al., 2005).

Differences Between Men and Mice

Therapeutic interventions based on an animal model of injury followed by a repair strategy simplify, by design, their representation of disease in man. To date, this limited experimental approach is still the best one available to neuroscientists and clinicians. Animal models permit the study of a neurobiological strategy in a more natural and complex setting than a tissue culture dish allows. Modeling a disease and treatment in one inbred species of rodent, however, will not predict its utility in clinical trials. For example, the most obvious difference between rodents and man is that the surface area of the brain of a mouse is 1/1000th that of the human brain. Studies of axonal regeneration in rats and mice consider an extension of axons for a few millimeters to a centimeter as exuberant growth that can

restore connectivity (Dobkin and Havton, 2004). Clinicians who try to interpret the results of animal models may not realize how tiny the rodent brain and spinal cord are compared to the human CNS. Axons in the human brain or spinal cord may have to grow from several centimeters to over a meter to reach neuronal targets. Neural progenitors may need to migrate 5 cm or more, not a few mm, to repopulate gray or white matter from the subventricular zone. Indeed, signaling molecules that fashioned the CNS during development managed far shorter tours of less than 1 mm for most projecting axons. A repair intervention in mice probably also requires a lot less regeneration of axons compared to the number of connections humans will need to produce complex behaviors such as goal-directed walking and manipulation of objects. Thus, the number of fibroblasts that secrete a neurotrophin or the number of implanted cells for remyelination that must migrate and reintegrate, for example, will likely be far greater and face more *in situ* obstacles in human subjects. In addition, the relative simplicity of structural networks that mediate testable cognitive functions in rats makes any anatomic reconstruction for a simple behavior in the rodent of unclear applicability to patients, who may have postinjury impairment of declarative and procedural memory and executive functions.

Aside from distance and network complexity, the immune and gene responses to injury and for repair in mice, rats, and humans are a work in progress; considerable differences in any key cascade will affect translational strategies. After SCI, for example, inflammation is less extensive in humans, although cytokine expression is similar, demyelination is probably less, Wallerian degeneration is greater over a longer time, Schwann cell responses are more pronounced, glial scar with chondroitin sulfate production is less extensive, Nogo-A is less likely to be in the periaxonal myelin sheath, and myelin-associated glycoprotein persists longer (Hagg and Oudega, 2006). Whereas regenerative endogenous processes such as axonal sprouting, remyelination, and activity-dependent physiologic adaptations within spared neuronal ensembles and pathways have been demonstrated in both rats, mice, and humans, the steps by which these unfold and the robustness of endogenous responses may differ.

In human trials, heterogeneity in genes, age, sex, medication taken, and premorbid health, severity of injury, precise timing after injury to onset of intervention, and other variables will differ from the perfect breeding and environment of animal experiments. These differences will not be overcome by using restrictive inclusion and exclusion criteria for randomized clinical trials or by increasing the sample size to power a trial. At best, clinical trials in SCI have limited the age range of subject enrollment to 14–18 years at the lower limit and 60 years at the upper limits. About one third of patients with stroke are less than 65 years old. Age 70–80 years is often the upper limit for eligibility in a controlled trial. Trials usually cannot enter enough subjects within a narrower age range. These human variables may confound in unexpected ways certain mechanisms of plasticity and regeneration.

Differences in Rodent Models

For repair research using small mammals, highly inbred rats or mice of the same species, strain, age, weight, and gender serve as the usual experimental population to increase the conformity of results. These small rodents are inexpensive, easy to handle and house, readily

trained, large enough for surgical manipulations, and lend themselves to standardized, but limited quantitative and qualitative functional tests. Mice offer transgenic models for highly specific studies of molecules involved in injury and repair. A single gene mutation permits the study of a specific phenotype, such as absence of an inhibitory molecule in the matrix of the cord or excessive production of a particular neurotrophin to stimulate a repair process. Males are used preferentially to avoid possible hormonal effects of the estrous cycle. The experiments are usually carried out on neonates, young adults from 3–6 months old or, occasionally, on elderly 2-year-old rats. The CNS of a neonate may allow far greater opportunity for molecular and morphologic adaptations than can evolve in an adult, although some models suggest that older age does not exclude the possibility of repair manipulations (Li et al., 2005; Markus et al., 2005).

Species differences can alter the result of an experiment. Different inbred murine strains may respond quite differently to ischemia or trauma and most mice respond differently than most rats in terms of injury and regenerative cascades. (Steward et al., 1999) For example, some rodents are much less likely than others to develop a glial scar after the same SCI that produces a large barrier to axonal regeneration in another species. Rats develop cystic cavities after a cord contusion whereas mice generally do not. Differences in genetic injury-induced immune responses or in the potential for endogeneous regeneration of neurons in inbred mouse strains, such as Nogo-A-specific knock-outs (Dimou et al., 2006), can account for wide variations in experimental outcomes for different rodent species and strains. Less obviously, different strains of rats may vary in important ways when morphologic, locomotor and sensory measures are examined (Webb et al., 2003). In SCI models, the choice between Wistar, Long-Evans, and Sprague-Dawley strains of rat has a great impact on the likelihood of locomotor recovery after the same spinal contusion injury (Mills et al., 2001). Skilled movements for manipulating food are supposedly highly conserved across species and genetically wired, but careful videoanalysis reveals that certain proper reaching movements with a forepaw have been breeded out of Fischer-344 rats. As a correlate, microstimulation of the motor cortex that represents the paw and distal arm reveals a much smaller representation for the wrist in some Fischer rats than in Long-Evans rats. Intensive training of paw reaching for food pellets may not enlarge the representation in the Fischer rats the way training affects representational plasticity in other rats, although training and other therapies can be constructed to do so (Conner et al., 2005). The number of trials that must be performed to expand the map may differ between rodent strains. Also, the quality of the forepaw strategy for pellet retrieval may differ between strains. Given the differences among animals, the researcher and clinician cannot assume that a human subject will have responses similar to any one rodent strain.

Preclinical studies of an intervention often do not include replication of the results of the same injury and repair model in different laboratories. The biologic significance of a manipulation would also increase if results were reproduced in another rodent species or strain. Replication in larger animals such as pigs or nonhuman primates further secures the biologic relevance, safety, and most feasible dose-response curve for clinical trials.

Differences in Injury Induction

Researchers are inclined to build upon an established acute injury model originally developed to produce a lesion of uniform distribution and volume to represent a partial equivalent of the human disease. A model for testing neuroprotective therapies may not serve studies for repair, however. Some injury models originally aimed to evaluate NMDA receptor-mediated neuronal injury in the cortex or hippocampus, for example. Most human lesions from stroke or TBI do not involve the hippocampus, unless global hypo-perfusion or hypoxia occurred. Of equal importance, human trauma and ischemia involve white matter destruction. The white matter is very thin in mice and only slightly greater in rats. The AMPA receptors of oligodendrocytes mediate cytotoxic injury, not NMDA receptors. Such basic variations across tissues help account for the failure of human clinical trials of interventions for NMDA receptor-mediated injury. Differences in receptor types within various regions of gray matter in the brain and spinal cord and primary versus secondary reactive changes that follow injury will also have to be reckoned with across rodent models in the translation to human neural repair.

Many models for induction of stroke have been developed, each with its peculiar characteristics. Scooping out cortex in an ablation model for stroke may produce the same volume and location of tissue destruction as a vascular occlusion, but the cascade of molecules, gene expression, and signaling that follow injury will differ between ablation and ischemia (Carmichael and Chesselet, 2002). The permanent middle cerebral artery occlusion model of stroke is more applicable than the transient MCAO model, because successful reperfusion generally does not occur in patients with the more seriously disabling types of stroke. For the study of specific repair mechanisms, the MCAO model may induce an infarct far greater in volume than most clinical strokes, reaching the depth of the lateral ventricle. This difference may affect signaling of the subventricular zone for neurogenesis. Focal gray-white matter and isolated small white matter infarcts may be more relevant for human repair manipulations (Carmichael, 2006).

Fluid percussion models of TBI often aim to produce moderate injuries that do not cause severe or lasting impairments (Bilgen, 2005). Only more pronounced deficits, however, are likely to be considered for initial trials of repair strategies in patients. Human TBI often includes cortical contusions of the anterior frontal and temporal lobes, diffuse white matter axonal injury (DAI), ischemia, bleeding, edema, and raised intracranial pressure, which may not be induced by a percussion model. Thus, repair strategies for the most important consequences of TBI, especially for DAI, cannot be attempted in most models.

SCI models include contusions induced by weight drop and electromechanical compactors that permit different severities of cord contusion, transection of the whole cord, hemicord or a tract by sharp axotomy, clip compression of the whole cord with variable duration and force, as well as ischemic and demyelinating models. The lesions induced by these methods may be reproducible, but none recreate the biomechanical compression of the ventral cord, shearing, deformity, and systemic complications that accompany human injuries. Sharp focal injury to a dorsal or ventral tract may be valuable for studies of methods to stimulate axonal regeneration, but probably causes too few changes in the milieu for too short a distance to serve as a preclinical model for human SCI repair strategies. Hemi-cord

sectioning models provide insight into potential biologic processes, but the results can be over-emphasized as to their applicability to paraplegic patients. For example, even though axon sprouting is reported, most investigators have not demonstrated that all of the axons in the tracts of interest had been damaged when behavioral recovery later evolved or that the sprouts arose from cut, rather than spared fibers. This confounder may account for the failure of recent experiments to reproduce the results of many early rodent experiments in which axonal regeneration was described distal to the SCI after local implantation of olfactory ensheathing cells or marrow stromal cells (Lu et al., 2006). Spared tracts within the site of injury or in remote pathways such as the intact homologous tract, as well as compensatory behaviors, can be responsible for functional improvements even when some regeneration is detected. Several approaches could increase confidence in the interpretation of these axonal regeneration studies. After behavioral gains plateau, the region of regeneration could be axotomized again to see if the deficit recurs. Or, a robust biologic process for regeneration would be suggested if reproduced by both a SCI and stroke platform or in two different SCI models. Nonetheless, differences in pathology, injury-induced cascades, gene expression, distances for migration and projection, and timing of interventions make treatments for repair in humans a great leap of faith when not built upon a thoughtful series of preclinical animal models.

REPAIR STRATEGIES

The variety of potential repair strategies and the aims of their effects are too numerous to try to consider the specifics of the delivery of any one intervention at the most utilitarian time and dose. Preclinical models, however, must try to solve this matrix if translation into a clinical trial is to be feasible. Preclinical testing should aim to set the conditions for meeting ethical standards for a subsequent phase 1 or 2 clinical trial. These initial trials are not expected to benefit an individual patient, but should offer a reasonable opportunity to document scientifically important and clinically relevant information. Preclinical models, then, must be designed to emphasize the search for safety, reproducible measures of efficacy, and possible applicability to the human condition.

Dose and Timing of Interventions

Since animal injury and repair models are unlikely to correspond to the complexity of human disease, the clinical researcher should at the least be able to give patients the equivalent dose of therapy (for a drug, the same measured plasma level) within the same therapeutic window of time. It is remarkable how often these simple criteria have not been met in going from the bench to bedside (Green et al., 2003; Anderson et al., 2005). Dose-response curves in pharmacologic studies are usually linear, J-shaped or an inverted U in which low doses and high doses work less well. When the dose used in preclinical trials causes adverse reactions in healthy controls or patients, the dose of a drug used for the trial may be reduced without going back to the model to assess the behavioral or morphologic response to a lower dose. Criteria also need to be established for the number of cells implanted in humans compared to the number in a rodent that will account for differences in the lesion volume and migration distance. The safety and dose, as well as the mechanism of repair, are best confirmed in a nonhuman primate model with a few animals.

For studies of neuroprotection, the timing of interventions in rodents is far more reproducible and often much sooner than possible in human trials. The timing of a repair intervention in human subjects will depend in part on the readiness of the milieu into which therapeutic cells and molecules are placed. Exposures that can confound or promote the intervention include inflammatory cells and substances, rapidly changing gene expression and signaling molecules, architectural barriers to migration or diffusion, and growth cone inhibitors. For example, gene expression for pro-growth and repair processes evolves over hours and days and differentially over the distance beyond the necrotic tissue (Carmichael, 2003). For the first 2–3 weeks after an experimental stroke, peri-infarct cortex expresses waves of neuronal growth-promoting genes, growth cone inhibitory proteins are less concentrated, many potential growth-inhibiting genes are not yet expressed, and molecular signals are present for the proliferation and migration of endogenous stem cells from the subventricular zone (Carmichael, 2006). Changes may continue for at least 3 months. After ventral root avulsion due to spinal cord injury, a large percentage of motor neurons gradually die over several months, unless neurotrophic factors are provided or the root is reimplanted near the ventral horn (Hoang et al., 2006). Thus, where the optimal timing has not been established, models should incorporate acute (<24 h), subacute (1–14 days), and more delayed (3–8 weeks and 12–24 weeks) experimental interventions within a moderate and severe model of injury.

Timing in the preclinical model could aim to parallel the point at which clinicians can state with some confidence that further measurable recovery of the targeted neurologic impairment in patients is unlikely (Dobkin, 2006). If the focus of restoration is on the affected hand, for example, by 4 weeks after stroke less than 10% of patients who still have no wrist and finger extension regain dexterity for grasping and pinching. The confluence of what is proceeding in the milieu of the injury to promote repair and the earliest time to predict futility for the clinical course of recovery, then, suggests that a biologic intervention for upper extremity function can be planned for 3–6 weeks after stroke as a first approximation. Recovery of walking is more difficult to predict than recovery of hand dexterity, probably because less readily measurable CNS and biomechanical adaptations enable walking despite marked loss of selective leg movements. After the most disabling hemiplegic stroke, the level of independence in mobility and self-care tends to reach a plateau by 12–18 weeks. Most models of stroke have not evaluated an intervention beyond one month after onset.

The spatial and temporal patterns of cellular responses and injury- and regeneration-associated gene expression vary for weeks and months after SCI as well. Most contusion models add a repair intervention by 2 weeks after onset. For investigators, perturbations of the severity of the induced injury may be of more experimental concern than varying the timing of a therapy (Himes et al., 2006). Most contusion models add a repair intervention by 2 weeks after onset. Clinical outcomes over time may be informative for modeling here, as well as for stroke.

The rate of spontaneous improvement after traumatic SCI is greatest within the first months after injury. The accuracy of predictions about maximal spontaneous gains in function improves as the length of time after the injury increases. Among patients examined within

72 h of SCI, roughly 25% with complete sensorimotor loss below the level of the lesion (graded by the American Spinal Injury Association score as ASIA A) will improve 1–2 levels below the lesion, but few will be able to walk by one year post-injury (Geisler et al., 2001). If patients are still graded ASIA A two weeks postinjury, only 10–15% will subsequently show spontaneous functional improvement by one year postinjury. If still ASIA A at 8 weeks, fewer than 5% will make some modest gains, mostly within one level above and below the SCI. Thus, for a randomized clinical trial of a repair intervention begun 8 weeks after SCI for subjects who persist as ASIA A with a cervical lesion, as few as 15–20 subjects would be needed in each arm to detect a 5–10 point increase in arm strength from 1–2 levels below the lesion (Steeves et al., 2006). If sensation to pinprick or touch is present below the injury level within the first 72 h to 4 weeks, even without apparent voluntary movement, about 50% of these ASIA B patients may achieve some motor function below the lesion (Geisler et al., 2001). These patients have some sparing of the spinothalamic tract, near the lateral corticospinal tract, so some conduction in the descending pathway may also recover as acute injury effects recede. However, if even useless but slight motor function is present within the first 72 h or several weeks after a cervical cord injury (patients graded ASIA C), useful motor function in the arms and legs evolves in most and 90% will walk on their own by 6 months (Dobkin et al., 2006a). Based on the Sygen Trial (Geisler et al., 2001) and the Spinal Cord Locomotor Trial (Dobkin et al., 2007), however, one can estimate that only 12–18 ASIA A and 24–35 ASIA B patients graded at 72 h are needed in each arm to power a randomized trial that aims to get about 30% more subjects walking with less than moderate physical assistance of a helper by 6 months after a cervical SCI. Thus, a therapeutic trial initiated within 72 h of injury will need to include a large number of subjects with clinically incomplete injuries, but perhaps 75% fewer ASIA A and B subjects, to detect a significant difference between experimental treatment and control groups. A subacute study can enroll still fewer patients to detect a potentially significant benefit.

For preclinical repair models, then, a contusion model that causes complete motor loss below the mid-cervical or lowest thoracic cord and persists for at least 8 weeks may best reflect the major inclusion criteria for patients who participate in the first Phase 3 trials of neural repair for SCI.

Site and Goal of Interventions

Lesions in preclinical models are most often generated in the rodent’s sensorimotor cortex or the corticospinal tract, which runs in the dorsal midline. Whereas this projection has an important role on the strength, dexterity, and speed of hand and foot and other multijoint movements in humans, most corticospinal fibers in rodents project to the dorsal horn, which is a sparse projection in primates, rather than to the ventral motoneurons (Lemon and Griffiths, 2005). In rats and mice, the tract most likely modulates the gain of segmental sensory inputs. Only equivocal brain–behavior correlations exist between the rodent and human supraspinal path, but the tract remains the focus of preclinical repair strategies. This seems reasonable, since a modest increase in the descending motor command in patients, even oligosynaptically via reticulospinal pathways or to propriospinal projections and then to spinal motoneurons (Mazevet et al., 2003), could have a clinically important functional benefit after a cerebral or spinal injury. The corticospinal tract also includes both ipsilateral

and contralateral projections from M1 and some of these fibers recross under the central canal of the spinal cord (Ralston and Ralston, 1985; Lacroix et al., 2004). In stroke models, an increase in dendritic spines has been reported in the unaffected M1 in response to greater use of the contralateral limb (Bury and Jones, 2002). The bilaterally spared descending fibers may sprout within the brain stem or cord after a supraspinal lesion or in the cord after a unilateral cord injury in nonhuman primates (Tuszynski et al., 2002) and perhaps in man. Most preclinical experiments in rodents have not looked for histologic or physiologic effects of endogenous or repair-mediated sprouting at bilateral cortical, brain stem, and spinal levels. With all of this potential plasticity, the locus for neural changes after a repair intervention and their behavioral consequences may not be fully detected to best understand and measure the effects.

The complexity of promoting axonal regeneration for clinically useful distances is formidable. The architectural barriers and the balance of pro-growth and inhibitory molecules in the milieu must be managed to guide axons past the lesion through white matter to their targets. Long ascending and descending tracts that are damaged by a cortical or subcortical stroke may be limited to axonal regeneration or sprouting to the nearby targets resulting in aberrant regeneration, which was found after a focal cortical lesion in monkeys (Dancause et al., 2005). Axon extension beyond the lesion site has been accomplished in select experimental conditions in animal models (Chen et al., 2002; Steinmetz et al., 2005). Therapeutics for complete SCI that act over short distances could target spinal gray matter immediately below the injury site. For example, a cell graft into a cervical lesion site that supports axon regeneration or remyelination may only need to extend several centimeters to reach 2 levels caudal to the injury to give a patient with C-5 tetraplegia considerably greater upper extremity use and independence in more daily activities.

Given the complexities of the signaling cascades that lead to functional circuits during development, attempts to reconstruct a neural network seem daunting. Implanted cells could serve as a synaptic bridge in the spinal cord or provide some level of excitation or inhibition within a disrupted intracortical pathway. Techniques to increase the efficacy of partially spared and intact circuits may be more practical. Preclinical models suggest that interventions to increase the concentration of certain neurotrophins, neurotransmitters, and other modulators involved in learning could enhance the effects of behavioral training and repair strategies (Conner et al., 2005; Ziemann et al., 2006).

Remyelination strategies for SCI have been proposed based on autopsy findings of intact axons that were unmyelinated in the surrounds of cystic lesions (Kakulas, 1999; Guest et al., 2005). Electron microscopy to confirm axon morphology and the length of demyelination distal to the site of induced injury has not been performed, however. Short distance remyelination has been induced at 7 days, but not 10 months after injury in rat spinal cord using human embryonic stem cell-derived oligodendrocyte progenitor cells, which confirms that both time and distance will be important variables to discern from preclinical experiments (Keirstead et al., 2005). Prior to human trials of a remyelination strategy, preclinical lesion models and human pathology studies must demonstrate that axon markers are present in the absence of myelin markers near a subcortical stroke or spinal cavitation.

The targeted axons should exceed at least a few percent of the tract's usual number of fibers if restoration of conduction is likely to lead to improvement in sensorimotor function.

Preclinical experiments in larger mammals and nonhuman primates ought to be considered prior to progressing from a rodent model to a clinical trial. Greater similarities between monkey and man, compared to rodents, include anatomic connections that underlie testable behaviors, immune and inflammatory responses to injury or to the repair intervention, the distances substances, cells and axons must be mobilized to reach around the injury and to repair targets, and perhaps the detection of tumorigenesis from cellular implants and malfunctional synaptogenesis. Brain structural and behavioral correlations across nonhuman primates, however, are not highly developed, so these studies may also not predict the effects of an intervention in patients. Insights from this larger preclinical model may still better prepare clinicians for the design of human trials. Without the requirement for statistically powering an efficacy study, a few nonhuman primates can provide insight about safety, dose-response curves, timing, and some confirmation about morphologic and behavioral effects (Oka et al., 2004; Yang et al., 2006).

Multiple Interventions

Combinational therapies in animal models have sometimes had a greater impact than single interventions on neuronal survival and axon regeneration (Nothias et al., 2005; Logan et al., 2006). The FDA requires each new intervention to be proven safe on its own. It seems likely, however, that if two short-term biologic interventions with a sound conceptual basis are found to be safe when given separately and in combination in both a small and large animal model, the two interventions could be approved for Phase 1 and 2 clinical trials.

Routinely taken medications may confound the effects on an intervention for repair in patients. The need for immunosuppressants or antibiotics after a cellular transplant may have adverse effects on regenerative capacity. Even the anesthetic given at the time of injury can alter inhibitory and excitatory neurotransmission for days in animals and in man. Patients take drugs for hypertension, diabetes, pain, seizures, and other ailments. These medications may alter the absorption, metabolism or bioavailability of the experimental intervention. Some may alter cAMP or neurotransmitter levels, including dopamine, serotonin, acetylcholine, and γ-aminobutyric acid, to possibly enhance or diminish learning and repair. These medications, as well as other acute and chronic disease processes, may alter the effects that had been found in the animal model. It will not be possible to eliminate patients from joining a trial based on the need for medications used to manage the disease at hand, so assays of the possible interactions of the experimental intervention and commonly taken classes of drugs are an important preclinical step.

Environment, Rehabilitation, and Postlesion Reorganization

Laboratory rodents do not hunt, seek mates, or solve the daily problems of survival. Most do not even interact socially in the same cages. Isolation can cause stereotyped behaviors. Dramatic consequences may also follow seemingly innocuous variations in daily rodent life, such as the amount of sleep, handling by the research staff, and caloric intake (Bruce-Keller et al., 1999). The responses made by animals depend largely upon rules that evolved from

interactions with natural environments. Deprivation may confound the interpretation of experiments and translation from animal models to human studies. The amount of stimulation and the opportunities to explore and learn provided by laboratory housing conditions can affect interactions between the environment and genetic factors, even leading to a reversal of gene effects bred into transgenic mice (Wurbel, 2001). The behavioral deprivation of standard housing conditions for rodents may be so stressful or so nonstimulating that any change in level of activity could alter gene expression in ways that are not likely in patients (van der Harst et al., 2003).

Exercise, environmental enrichment that permits activity in cages, and skills training enhance synaptogenesis, neurotrophin levels, mechanisms of learning, and recovery in most, (Johansson and Belichenko, 2002; Vaynman and Gomez-Pinilla, 2005) but not in all models of brain and spinal cord injury. It is less certain that these effects would be found in wild animals living in their natural habitat or in patients. Rodents in natural environments evolved to find food and shelter by scurrying about as they vigilantly attend to details in their surroundings. Aimless extreme exercise, such as running on a treadmill or in a wheel for 5000 rotations, may be much less of an inducement for neurotrophin production in people. Skills retraining may be more likely to induce neurotrophins, long-term potentiation, and synaptic efficacy.

One boost for exercise and training as an adjunct for repair strategies evolved from studies of hindlimb step training on a treadmill in cats and rats after a low thoracic spinal cord transection. Experiments revealed that polysynaptic circuits, perhaps including central pattern generators, learned to produce alternating flexor and extensor movements, leading to hindlimb stepping (Barbeau and Rossignol, 1987; de Leon et al., 1998). The animals did not walk over ground. The results led to the possibility that similar training could improve walking in patients with complete and incomplete SCI and stroke. The model, however, is for quardrupedal stepping on a moving treadmill belt, not necessarily for bipedal walking with an upright trunk. Supraspinal inputs to the lumbar motor pools are necessary for patients to walk (Ivanenko et al., 2005). Also, few preclinical studies compared treadmill training with no specific rehabilitation to examine spinal learning and pattern generation in mammals after incomplete SCI or cerebral stroke with particular descending tracts spared. Thus, the translation of the strategy to patients is perhaps less direct than is often suggested (Wolpaw and Tennissen, 2001). Treadmill training with partial weight support by a chest harness attached to an overhead lift or by robotic assistive devices to step the legs offers a system to provide phasic proprioceptive and cutaneous inputs for step training in patients (Dietz et al., 2002). This approach allows repetitive practice, but just what a subject is supposed to be practicing and learning to improve gait often goes undefined. To date, translation of the spinalized rat and cat model to human subjects with complete or incomplete SCI has not led to better outcomes in controlled randomized clinical trials with blinded outcome measures (Moseley et al., 2005; Dobkin et al., 2006a). Thus, even this rather well-defined model of injury, training and recovery has lagged in its translation to patients.

Interventions for repair in animal models have increasingly added a training paradigm to try to optimize behavioral outcomes and exercise or training-induced plasticity to augment the

regenerative intervention (van Praag et al., 2000). The optimal dose of endurance exercise after stroke appears to vary by intensity and duration of exertion, region of the brain, and by production of different growth factors (Ploughman et al., 2005). The optimal intensity and duration of therapy to maximize gains after stroke or SCI is rarely examined in animals or prior to undertaking clinical trials (Dobkin, 2005b). Skills training, procedures to make practice progressively more difficult, and reinforcement paradigms to maximize learning are rarely considered in animal and human studies. Indeed, most studies of augmented practice during rehabilitation for stroke, for example, have offered about 16 h of additional treatment for a specified problem in patients with mild to moderate impairments or disabilities; these trials have revealed an average of only 10% improvement with the additional treatment time (Kwakkel et al., 2004). The intensity of task-related training that aims to incorporate the cells, axons, dendrites, and synapses of a partially regenerated pathway is no less important to the success of a regeneration strategy than the type and timing of the repair intervention.

Outcome Measures

Clinicians use systems-level outcomes. In the rodent, rather limited behaviors are tested. Physiologic, histologic, and molecular markers may serve as surrogates for behavioral outcomes in preclinical models to assess the biologic activity of the repair intervention. Gains in the targeted behavior are most important, however, before proceeding to clinical trials. Many functional tests have been standardized for forelimb, hindlimb, whisker sensation, and learning (Whishaw and Kolb, 2004; Nichols et al., 2005). Behavioral gains in animal models may intuitively suggest the potential for parallel sensorimotor gains in humans. Behavioral tasks used in rodent tests, however, have very limited ethnological relevance to laboratory rats and mice or humans. The interpretation of functional gains in quadrupedal locomotion or reaching for a food pellet is complicated by not fully knowing the anatomic tracts necessary to perform the activity. Indeed, remarkably few studies in rodents have correlated brain–behavior relationships (Gharbawie et al., 2006). A change in behavior, especially for the simple behaviors that are tested by ordinal scales in rodents, may allow comparisons with controls, but does not allow an investigator to extrapolate to the response in humans. Several frequently used behavioral tests leave much to be desired for translational studies.

In many injury models, the Morris water maze serves as the primary measure of motor and spatial memory outcomes (D’Hooge and Deyn, 2001). This place task requires the rodent to find a partially submerged platform in a water bath by using visual cues outside the tub. The task is not without built-in biases, however. Some rodent strains are better in their visuo-spatial memory than others (Van Dam et al., 2006). These differences may affect their vulnerability to the effects of a stroke or trauma and their subsequent gains. The history of reinforcement also contributes to spatial performance. For example, animals trained with the platform present in 100, 75, or 50% of trials behaved differently (Gonzalez et al., 2000). The 75% group had impaired spatial acquisition and the 50% group failed to learn even when the animals were placed on the platform after an unsuccessful trial. After a hippocampal lesion, rats are not supposed to be able to remember how they had navigated a maze. If some salient feature in the maze, a landmark they perceive, gives them positional information, they can learn to use this to find their way, however. The strategy is a rigid one in that they will not

reproducibly find their way in another maze that has no such landmark (Ramos, 2002). Thus, the outcome measure of a treatment may be confounded by genes, prior experience, side effects of experimental interventions, modest differences in schedules of reinforcement during learning, and other less obvious factors.

A neurological outcome measure showing that injured rats perform better after an experimental intervention compared to no treatment may be statistically significant, but the difference may not be behaviorally significant. Preclinical studies should examine both the statistical significance and the clinical significance of changes in behavior. In human trials, the amount of gain must entail a clinically meaningful change in impairment and disability. Most analyses of animal experiments do not perform an *a priori* power analysis to optimize the sample size and may excessively rely on *p*-values. A *p*-value does not suggest how large the effect is or whether the result is clinically meaningful. Experiments would be easier to interpret if effect sizes and confidence intervals were presented. A preclinical study, for example, that reports a significant difference in the time to find the platform in a water maze in one treatment group compared with another as a less than 2-s difference for an 8-s task will leave the clinician wondering whether this is a robust enough effect to justify a Phase 1 trial. A change in the observed kinematics of hindlimb stepping using the 14 visual descriptors from the BBB locomotor scale, the most frequently employed outcome measure in rat SCI studies, may appear impressive statistically, but the neural organization for different levels of the BBB is not known (Webb and Muir, 2005), so investigators cannot conclude that a repair strategy had a particular biological effect. If an animal recovers a foot placement reaction, does that mean that a particular pathway must have been restored? Is this relevant to bipedal walkers? How can one interpret the seeming divergent but not uncommon finding that less asymmetry of forepaw use is found after a stroke repair intervention, but skilled reaching for food pellets is no different than in the control group? These brain–behavior correlations are no less uncertain in large animal models such as pigs and nonhuman primates (Lemon and Griffiths, 2005). A clinician would have more confidence in some of these brain–behavior correlations if regenerated axons were shown to make synapses with their targets and subsequent ablation of the axons reinstated the behavioral deficit.

In animal experiments, neuroanatomical tracing techniques to determine the number of regenerated axons or proliferated stem cells, as well as assays of the concentration of particular proteins and other substances, may reveal statistically significant differences compared with a control intervention. These outcomes may seem to be foolproof compared with behavioral measures, but they too have inherent problems. After injury, some histologic and molecular changes may be reactive rather than pathological. For example, shrinkage of neurons after axotomy may not imply the evolution of irreversible cell apoptosis (Kwon et al., 2002). Many of the changes in expression of mRNAs, proteins, genes, and trophic and tropic substances in neurons, glia, and in the milieu of injury that suggest components of a repair process may simply be reactive changes. Whereas these substances had been vital during development, they may not necessarily serve a critical function for axonal regeneration in the adult. Thus, a focus in preclinical experiments on one signaling molecule

may make for interesting neuroscience, but may overstate or understate the need to regulate that molecule in human studies.

Controversies surround the matter of how to best stain and count the cells, axons or boutons within three-dimensional space in serial tissue samples to demonstrate repair processes. If, for example, investigators want to know the number of new axons regenerated, they must follow axons as they weave in and out of multiple serial sections. If they want to know the number of neurons that migrated through brain tissue after transplantation, they must sample far and wide in sections not much thicker than those neurons. These anatomical studies require multiple staining techniques, digitalization, camera lucida, and reconstruction methods. Electron microscopy is necessary to quantify neosynaptogenesis. Static images may not reveal other insights into biologic activity, such as axon retraction and extension in living organisms (Zhang et al., 2005). In addition, repair studies focus attention on a particular region of interest, but may not look for similar changes in other regions that also would explain a change, for example, in forepaw skills. The clinician may misinterpret the enthusiasm of the neurobiologist for the measurable change without realizing that the model has not explained whether a behavioral gain can be attributed to the intervention. Preclinical experiments that assess a broad range of markers over time and over the course of behavioral gains would better convince clinicians that a particular experimental intervention is essential to trigger a critical cascade for repair.

Physiological studies in animal models provide insights for human studies. Functional magnetic resonance imaging, positron emission tomography, diffusion tensor imaging of axons, evoked potentials, optical intrinsic signal imaging, and other methods could be used in parallel in animal and human trials to explore residual connectivity, the best site for a repair intervention, monitor remyelination, determine whether the motor network is engaged by task-specific rehabilitation, and monitor the dose of therapy needed to maximize cerebral reorganization after a biologic therapy (Dobkin et al., 2004; Dobkin, 2005a; Carey et al., 2006; Lotze et al., 2006). Several studies of rehabilitation interventions for the upper extremity and for walking monitored by repeated fMRI or transcranial magnetic stimulation studies at regular intervals during therapy suggest that this approach may give insight into gradually adapting brain–behavior and dose-response relationships (Koski et al., 2004; Dobkin, 2005b; Dong et al., 2006).

Basic research in developmental neurobiology, animal models of CNS and PNS injury, and animal and human studies of neural plasticity offers the promise of being able to augment rehabilitation interventions for the most impaired patients by translating neural repair strategies from the bench to the bedside. Preclinical studies can draw upon the expertise of basic and clinical scientists and rehabilitation experts to inform the design of clinical trials.

Acknowledgments

Contract grant sponsor: National Institutes of Health; contract grant numbers: NINDS T32 HD0741, NICHD HD39629, NICHD RO1 HD06740, NICHD R24 HD050838.

Contract grant sponsors: Dr. Miriam and Sheldon G. Adelson Medical Research Foundation.

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Table 1

Experimental Phases for Clinical Trials in Patients

Phase 1	The first stage of clinical testing assesses the safety, tolerability, pharmacokinetics, pharmacodynamics, and maximum tolerated dose of a therapy in healthy and affected subjects. Dose-response safety curves are developed. The number treated is too small to assess efficacy.
Phase 2	The first clinical studies aim to gather preliminary data on the effectiveness of the experimental therapy by testing one or more doses in a modest number of subjects. The design for these trials also assesses the feasibility of the inclusion and exclusion criteria for participation (location of lesion, age, gender, time after injury, severity of deficits, other medical complications, concomitant medications, ability to give informed consent, family support, etc.); the utility of chosen outcome measures; and the likely robustness of the intervention to help determine the number of subjects that will need to be treated successfully to best power a larger trial. Phase 2 studies test the standardization procedures that have been adopted. Trials include a control group that receives a specified intervention (such as a placebo medication, manipulation or physical therapy) to equal the experimental group's intensity of care, blinded outcome measures, and external monitoring by a safety committee for short-term adverse reactions and risks.
Phase 3	Subjects with the disease and entry criteria of interest are randomly assigned to the experimental or control intervention in sufficient numbers to reject the null hypothesis that the experimental treatment is no better than the control therapy. The design and management of the trial are performed so as to eliminate bias - random allocation to a placebo, sham or other control intervention, predefined primary and secondary outcomes for statistical analyses, masking at least the person who conducts the primary outcome measures, and the subjects and investigators when feasible, a data management group that receives and analyses all data independent of the investigators, and a safety monitoring committee that evaluates risks, benefits, and ethical issues as the trial proceeds.
Phase 4	Postmarketing safety surveillance collects voluntarily offered data sent to the commercial company that licenses the intervention. Formal studies may add information about the longer-term risks, benefits, and optimal indications for use of the approved treatment.