Manuscript title

Can Non-human Primate Experiments Expedite Translation of Potential Reparative Interventions After a Spinal Cord Injury in Humans?

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Abstract

Progress continues in developing reparative interventions to enhance recovery after experimental spinal cord injury (SCI). Much of the progress has been made with rodents, but they differ in some important ways from humans and other primates in size, neuroanatomy, neurophysiology, physiology, biochemistry, immunology, and behavior. Questions discussed were to what extent SCI rodent models present limitations for ensuring the efficacy and safety of a treatment for humans, and under what circumstances it would be advantageous or necessary to test treatments in non-human primates before or as an adjunct to clinical trials in human patients. We focus on the recovery of skilled motor control, which enables us to compare and contrast the known differences in the organization of the motor systems and in the behavior among rodents, non-human primates, and humans. In addition, we point out critical issues related to safety in the context of promoting neural connections after an injury that could lead to malfunction. Non-human primates and humans share a myriad of similarities between the structure of their motor systems and motor behavior. Therefore, the non-human primate SCI model provides many unique advantages for testing experimental effects and understanding the safeness of a reparative intervention to promote functional recovery following SCI with the appropriate relevance for humans. We conclude that non-human primate studies are critical for the timely and safe translation of selected potential interventions designed to repair neuromotor impairments in humans.

Introduction

Traumatic spinal cord injury (SCI), along with other chronic paralyses, has long-term health, economic, and social consequences worldwide [1,2]. The physiological, psychological, and social hardships of individuals with SCI as well as the enormous socio-economic costs demonstrate the urgency to minimize the impact of paralysis and maximize the level of functional recovery. Treatments that lead to partial functional recovery, and thereby greater independence, can significantly improve the quality of life of individuals following SCI. Consequently, there is considerable need to apply to the human those interventions that have shown effectiveness in promoting functional improvement in laboratory animals.

Progress continues in the identification of interventions that show promise in augmenting CNS plasticity after injury by promoting axon regeneration and sprouting after experimental SCI in non-primates, especially rodents [3-6]. Several of these treatments may be efficacious in human patients with SCI, some of which have or are entering in phase-1 clinical trials. Important differences exist, however, between rodents and humans including body size and design, neuroanatomical organization, electrophysiological properties, function of the descending tracts, and the repertoire of motor behaviors, as well as inflammatory and immunological responses.

This position paper points out disadvantages of the commonly used rodent models for ensuring the efficacy and safety of SCI treatments for humans, and addresses how the use of non-human primates can play a part in the development of therapies for humans with paralysis, and facilitate the successful advancement of potential treatments to clinical trials. We focus on motor performance since this behavior is well-studied and has important similarities in the organization of the neural systems controlling movements between some non-human primates and humans. A similar examination of autonomic and other neural functions compromised after SCI also would be useful for a more comprehensive strategic approach in optimizing functional recovery after SCI. Critical issues related to the safety of a potential intervention are examined. It is concluded that non-human primate studies can probe the effects of neural plasticity on perceptual, cognitive, and emotional function with a refinement that cannot be reached in rodents. Specific examples illustrate some advantages of translational SCI investigations in non-human primates. Although the focus of this document is on SCI, given the common elements involved in a wide range of neurological disorders that impact the sensorimotor system, the conclusions of the present position paper extend beyond SCI.

I. Differences in anatomical and behavioral features between rodents and primates (humans and non-human primates)

A. There are critical differences in the organization of the sensorimotor systems between the rodent, non-human primate, and human

Whereas there has been a remarkable conservation of many characteristics of vertebrate sensorimotor systems across species, some features of the motor systems at the molecular, cellular and systems levels have undergone pronounced evolutionary changes [7,8]**.** This is particularly true for the unique mammalian component, the motor cortex and its descending outflow, i.e., the corticospinal tract (CST) that projects extensively to the brainstem and the spinal cord in primates. In many primate species, the CST can influence motoneuron activity both directly and indirectly [8]. Indeed, the evolution of human and non-human primate species includes a massive increase in the relative size of the neocortex and the amount of neocortex giving rise to the CST, the appearance of a fast-conducting component of the CST, and the migration of corticospinal axon location from the dorsal column to the dorsolateral spinal cord white matter [9]. Differences between rodents and primates in the pattern of CST terminations are both qualitative and quantitative. In rodents, the CST mainly projects to the dorsal horn neurons and premotor spinal circuits. In many non-human primates, such as the rhesus monkey (Macaca Mulatta), the projection pattern of the CST is much more complex; a significant portion of the CST fibres also project to the ventral horn and some axons synapse directly onto motoneurons [8], in particular those innervating hand muscles. In humans this trend is yet more marked [10]. Stimulation of CST neurons in the motor cortex evokes motor responses that significantly differ in primates compared to rodents [11], as well as between different primate species [8].

Such development of the descending motor pathways from the brain provides the CNS of primates with the capacity to perform fractionated recruitment of motoneuron pools innervating distal muscles, and contributes to an increased capacity to control the hand musculature [11-13]. In particular, the appearance of direct cortical projections to spinal motoneurons correlates with the emergence of precision grip control between the thumb and the index finger, which only exists in some primate species [8]. Accordingly, unlike in rodents [13-15], interruption of the cortical projections to the spinal cord provokes a major impairment in fine motor function with the hand and foot in primates, the magnitude of which increases further in the human [16]. Likewise, a lesion of the CST has little effect on overground stepping in rodents [17], indicating that the motor cortex is not an essential structure for creating the muscle synergies sustaining simple locomotion in rats and mice. On the contrary, damage to the CST in the spinal cord provokes some permanent deficits during stepping in rhesus monkeys [15,18], and leads to a motor impairment of the human lower limbs that is severe enough to compromise independent walking [16].

Fine motor control of the forelimb can be tested in rodents and is affected by lesions to the CST [19]. The finesse in digital control, however, is far less in rodents than in non-human primates. The neural circuitry underlying fine motor control, and possibly locomotion, differs both in nature and function between rodents and primates. Although unproven, testing in non-human primates will likely provide a better prediction of the potential of a spinal cord therapy to mediate recovery of manual dexterity, and to some extent stepping, in humans. This viewpoint is based primarily on the substantial differences in the projections of the CST to the motor pools of the distal forelimb and hindlimb muscles between rodents and non-human primates. Furthermore, there are marked differences in the musculoskeletal design of the forelimb, hand, and distal digit musculature in primates and rodents.

Differences in motor behavior and the underlying organization of the neuromotor system suggest that strategies promoting regeneration of the CNS might influence neuronal systems of rodents differently than those of primates, resulting in divergent anatomical and functional outcomes. Given these differences, even if there are similar outcomes**,** the neurological basis for the improvement may differ substantially. For example, compared to rodents, primates engage more complex neural circuits in the parietal and frontal lobes of the cerebral cortex even for the simplest of skilled movements. This reliance on the cortex for motor function, in turn, may offer a greater plasticity and recovery following a partial SCI. Sprouting of spared CST fibres onto the descending brain stem motor systems and associated changes in the structural organization of the cortex may provide a unique capacity for plasticity that can lead to significant improvement in motor function, particularly if fibre growth can be enhanced experimentally. Treatments that minimally promote plasticity in rodents might be considerably more efficacious in non-human primates and in humans. Although technically challenging, there is a clear need to document changes in the cerebral cortex [18,20-22] as well as responses of non-CST descending pathways when a treatment is applied to primates with a SCI. This potential for re-organization at multiple sites in the brain following SCI may be an effective means to enhance functional motor recovery in response to incomplete injuries [23]. Similarly, to what extent could regeneration of a few ascending fibres across the injury site restore sensory function and contribute to improved motor behavior? These possibilities could not be tested with the same resolution in rodents since sensory discrimination does not appear to be as critical for manual dexterity in rodents compared to primates, nor is the response to injury of the sensory area in the brain similar[24].

There also are substantial differences between rodent and most primate species in the distances over which neural systems project, or might be required to regenerate or sprout after an injury. This difference potentially limits inferences of plasticity or regeneration studies from rodents to primates. This is relevant for injury to the cervical spinal cord, but may be even more problematic for re-innervation of the lumbar regions owing to the long distance to reach locomotor circuits in humans. It is important to note that even limited sprouting or regeneration in the cervical spinal cord associated with recovery of some aspects of the fine motor function can be extremely beneficial to humans. There are a number of differences in size, as well as organization of the neuromotor infrastructure and underlying functions, between primates and rodents that could result in different outcomes in rodents

and primates. Non-human primates provide a model of human psychomotor interactions that has no equivalent in rodents—interactions that range from cognitive mechanisms of motor use and adaptations to the effects of motivation and affect (e.g., frustration, depression) on motor behavior**.** Thus testing potential treatments in non-human primates would give a better prediction of the effects of any treatment on sensorimotor systems in humans than can be obtained in rodents.

B. Hand motor functions in non-human primates are similar to humans

Detailed assessments of motor capacities and neuromotor connectivity can and should be thorough and comprehensive in non-human primate translational SCI studies. From the perspective of clinical trials, a significant advantage of testing motor performance in non-human primates is the greater similarity in functional measures with humans. Indeed, the precision grip, pre-shaping of the hand, grasping, and other manual prehensile tasks performed by rhesus monkeys and various other Old World species are very similar to these behaviors in humans. On the other hand, testing of fine motor control in rodents remains limited to a coarse success/failure rate assessment. In rare cases, time-consuming video analysis is used, but the detailed fine control of the distal phalanges in rodents remains rudimentary at best [19]. Moreover assessment of cortical connectivity, supraspinal access to spinal motoneurons, and segmental circuit properties can be performed similarly in non-human primates and humans. Therefore, evaluation of post-lesion motor behavior should include careful documentation of how a range of different manual functions are performed over the course of the recovery period. In particular, combined analyses of motor pool recruitment patterns and kinematics of the head, trunk and limbs can provide decisive information on the degree to which the animal recovers using compensatory strategies to perform the task successfully. Transcranial magnetic stimulation (TMS), magnetic resonance imaging (MRI) of the brain, and recording of sensory-evoked potentials can provide additional tools for obtaining very similar motor performance related data in non-human primates and humans.

Behavioral and physiological assessments, however, should not be limited to manual dexterity. It is important also to assess stepping ability and other key motor functions. The extent to which stepping and grasping differ in their underlying neural processes and their intrinsic capacity to recover from a SCI in the primate are not fully understood [15]. There may be a unique capability in non-human primates in that engaging the neural circuits for stepping may facilitate the recovery of timing in motor pool recruitment following injury, and thus promote restoration of fine motor control [25]. There is also the option of studying quadrupedal or bipedal walking. In addition to quadrupedal locomotion, many primate species practice bipedal walking with characteristics close to human walking, and bipedal stepping on a treadmill or overground can be tested and quantified [26]. Furthermore, manually- or robotically-assisted bipedal step training following SCI can be implemented in non-human primate subjects.

C. Other neurological systems present critical differences between the rodent, non-human primates and human

Although the present focus is on neuromotor function and the safety of interventions designed to improve function following a SCI, other areas of function should be examined similarly with respect to the advantages and disadvantages of using non-human primates relative to the commonly used rodent models. Failure to maintain a critical level of function in all physiological systems is life threatening. Methods to improve recovery of autonomic functions are ranked as very important by injured patients [1]. The overall architecture of the autonomic neural pathways is similar among mammals. Nevertheless, human bladder control and sexual function is more similar in non-human primates than rodents. Issues of autonomic control, such as blood pressure changes when assuming a vertical posture after a SCI also are important, and non-human primate experiments are likely to provide a better predictor of treatment effects than those in rodents.

II. What advantages do non-human primate models have compared to rodent models when testing therapeutic interventions?

Rodent and feline models have been used for the development of the current treatments that are under consideration for use after a SCI, and they have to remain the mainstay of experimentation. However, testing some forms of treatments in non-human primates before undertaking human trials is likely to provide essential information on the efficacy as well as on possible adverse effects of specific treatments. A major advantage in using a large non-human primate is the technical capability to comprehensively examine a range of highly skilled motor functions of the hand, including hand-to-eye coordination using electrophysiological and biomechanical tools. Furthermore, it is feasible to monitor the degree to which, and how, different neuromuscular components contributing to the wide range of hand functions. Based on extensive degree of activity-dependence on motor function following a spinal cord injury in mice, rats, cats and monkeys, documentation of these activity patterns could play an important role in interpreting the efficacy of any interventions being tested[27,28].

Careful daily monitoring of health related variables, such as appetite, weight gain, immunological status, etc., can provide crucial information for interpreting the results from a given experiment. The general experimental strategy should be to obtain as comprehensive a database as possible from each subject, thereby enabling a more accurate interpretation of the results. In addition, the number of animals used has to be sufficient to reach statistically sound outcomes. Given the inherent variability of spinal lesions as well as individual anatomical and behavioral differences regardless of the species, every effort to pair experimental and control subjects with a respect to size, age, behavior and general health should be made. A comprehensive battery of highly quantitative assays also can minimize the number of animals necessary to demonstrate the statistical significance of treatment effects. A recent example of an experimental design incorporating these principles in testing the effects, as well as the

safety of the procedure of administering NOGO-A specific antibody in a non-human primate on functional and anatomical recovery following a SCI was recently reported[14]. In this case, reasonably convincing evidence of the effectiveness of the intervention was based on three pairs of monkeys combined with data from two other experimental and one other control monkey.

A. Can non-human primate research facilitate translation of therapies to humans?

In deciding whether a potential intervention should be studied in a non-human primate rather than in rodents, a decisive factor may be whether greater progress and/or rate of progress can be made towards more effective therapies for humans. Perhaps as important, however, is the question "to what extent can the efficacy and safety of the treatment be assessed in rodent studies"? The answer to many important pragmatic questions that can be addressed effectively in the rodent can improve the probability of successful extrapolation to humans. Examples of some of these questions are: "which types of cells should be implanted?", "how many cells should be implanted?", "what percentage of cells survive after implantation?", "what should be the postoperative procedures to maximize the effectiveness of the implantation?", "how much and when should a specific growth factor be administered?", and "what specific types of immunological, urological, respiratory, sensory and motor effects might be expected and over what timeframe?" After answering some of these questions, however, studies in non-human primates would provide a more accurate prediction of the optimum treatment procedures to use in humans. The number of critical variables that can affect the outcome of an experiment will be even greater when multiple interventions are tested in the same subject. In these cases, combined repair strategies are likely to be more successful if first studied in non-human primates. Experiments in non-human primates, while more expensive and cumbersome, and technically, more complex than those in rodents, remain far less expensive than clinical trials in humans. Trying to optimize these methods in human subjects would also be prolonged by the limited number of patients available for trials, and would be highly problematic ethically. The financial cost associated with a single human clinical trial could support several primate studies, and the latter, arguably, would lead to greater and more rapid advances in our development of treatments after a SCI.

Whereas the non-human primate model provides clear advantages, a general recommendation for all interventions to be tested in non-human primates might impede the development of some treatments from rapidly reaching the clinic. Almost all experiments with non-human primates are challenging for multiple reasons. There are a limited number of laboratories that have the necessary skills and resources to carry out experiments that can examine all of the systemic functions affecting the treatment being tested. The cost of these studies compared to rodents will be high in testing newly evolving ideas in a timely manner. A potential solution is the strategic formation of appropriate collaborations among investigators having complementary skills and resources. To achieve this end, funding agencies must recognize that multi-laboratory strategies will be necessary if the non-human primate model is to be used with maximum effectiveness and efficiency.

B. Advantages of demonstrating the safety of potentially effective invasive interventions in a non-human primate for treating SCI in humans

Most SCI patients are young and will attain a normal lifespan. After rehabilitation most will become active and productive members of society. Trials of therapies for SCI must take this into account. Although patients are severely disabled and the need for a treatment is urgent, the condition does not cause a rapid and inexorable decline leading to death. Some potential treatments for SCI carry a risk of enlarging the area of damaged tissue. Because extending the level of an injury by even half a spinal segment in the cervical region of the spinal cord can have adverse functional consequences for patients, absolute confidence that the treatment to be delivered will not cause additional damage is essential. Increasing the amount of injury at the mid-thoracic level, by contrast, has fewer functional consequences. There also are unique safety factors for those who have complete rather than incomplete lesions. Testing in non-human primates affords an efficient alternative with greater access to histological, morphological, and other information. Invasive therapies such as cell transplantation or the administration of growth factors or agents to neutralize neurite growth inhibitors that are tested first in a non-human primate model could help insure faster, safer and more efficacious use in humans with SCI [14]. Prior studies with non-human primates might avoid premature exclusion of a potentially useful intervention, and at the same time enable a safer design for a clinical human trial.

Most of the therapeutic strategies for SCI target neurons and their growth capacity. Axonal sprouting and neural regeneration, however, can lead to malfunction such as neuropathic pain which could be more difficult or even impossible to test behaviorally in rodents, but quite feasible in non-human primates. Some information, however, can be gained from rodent studies, e.g., aberrant axonal sprouting associated with allodynia-like hypersensitivity of the forepaws has been reported following intraspinal graft of neural stem cells in a model of rodent SCI [29]. Through extensive interactions between the therapist and the non-human primates, and thorough analyses of non-human primate data, it is possible to monitor the safety, side effects, pain responses, neuropsychiatric behavior, bladder and bowel function, sexual function, autonomic dysreflexia, and other functions after an intervention. Each of these can be crucially important after a SCI as almost all patients at some time is confronted with life-threatening complications. Likewise, inflammatory and immune responses differ in kind and extent between primates and rodents [7] and could contribute to altered secondary cell damage, removal of debris after trauma, axonal plasticity, and eventual recovery. These differences could render a neural intervention that was beneficial in rodents to be ineffective in primates. These factors suggest that potential interventions can be tested more stringently in non-human primates than in rodents while providing quantitative assessment of efficacy. In effect, these results could critically enhance the safety of the patients participating in a clinical trial.

C. The benefits of critical translational studies in non-human primates outweigh the costs of the animals involved

There are continuing discussions in many countries and at individual institutions regarding the conditions under which non-human primates can be used in research. For example, in a recently published report [30], the UK's Medical Research Council and the Wellcome Trust concluded that although there is a need to review the ethical and scientific justification for primate use and strict legal controls, some biomedical problems are such that alternatives are not available or appropriate and it is important to conduct research on non-human primates.

Our present analysis is focused on the cost/benefit ratio of experiments in non-human primates with the purpose of improving function post-SCI within the framework of the current national and international standards for the use of experimental subjects. This analysis also is done with the recognition that experiments should be performed only if there is no other way of obtaining the results or if, as judged by the appropriate governing bodies, the benefits of the work to humankind outweigh the costs of the animals involved. Based upon differences in the organization of sensorimotor systems between rodents and primates as well as the safety factors involved, we concluded that the limited and optimized use of non-human primates, such as rhesus monkeys, can be highly beneficial in efforts to improve treatment efficacy and safety in humans following a SCI. At present there does not seem to be any good scientific rationale for carrying out SCI research in apes (bonobo, chimpanzee, gorilla and orangutan).

III. The type of non-human primate and SCI are important factors to consider

A. Rhesus monkeys have the most similar known motor system to humans

The complexity and refinement in the organization of the cortical and spinal circuitry underlying motor behavior have increased gradually during primate evolution, i.e. from New World and Old World monkeys to apes and humans. Accordingly, the non-human primate to be used would depend on the specific questions and paradigms to be studied. However, based on experience to date, and on the amount of neurophysiological and neuroanatomical data available, the rhesus monkey, an Old World monkey, provides a clear advantage in translation of findings to humans. Notably, owing to its relatively large size, the rhesus monkey is more comparable to humans with respect to metabolism, dose response, time frame of treatment, drug uptake and half-life, and blood brain barrier properties. Moreover, the Old World rhesus monkey, as opposed to New World marmoset, squirrel, and spider monkeys, presents the advantage of being easily trained to use the hand, presumably because their projection patterns of the CST (including direct connections with motoneurons) and non-primary motor cortical areas are closer to those of humans. These animals also possess the cognitive capacity to

learn and to perform the assessment and therapeutic tasks that parallel those used with humans. Indeed, historically, they are the monkeys of choice for all studies on single-unit recordings during fine motor control movements, as well as brain-machine interface investigations. Consequently, when investigating the potential of neural repair interventions that promote recovery of fine motor skills after a SCI or other debilitating condition, rhesus monkeys are better suited than other non-human primates as an animal model for humans [8].

B. Each type of spinal cord lesion has experimental advantages and disadvantages

The type of lesion studied should depend upon the specific experimental question. SCI studies generally can be categorized as (1) contusion, (2) anatomically incomplete transection, and (3) anatomically complete transection. Each of these SCI models offers advantages and disadvantages which should be taken into account when designing translational investigations in non-human primates. In any case, it is essential that the lesion model induces a permanent and reproducible deficit in at least one area of sensory, motor or autonomic function. Otherwise the model cannot be used to test effective interventions.

(1)Because most human SCI results from trauma as opposed to partial or complete transection, the contusion models are considered to more closely resemble human spinal cord damage, compared to a transection injury. This model can be a reproducible, with predictable and consistent functional outcomes that allow testing of potential therapies, particularly those that mitigate the formation of lesion cavities and enhance tissue sparing. Contusion injuries also provide a model to test the effect of transplanting cells or materials to fill the lesion site. Ischemia and cavity formation as well as the partial tissue sparing associated with such lesions, however, add significantly to the complexity of dissecting the underlying mechanisms associated with functional improvements following therapeutic manipulations. When the goal is to investigate specific neural mechanisms underlying therapy-mediated recovery or to distinguish between spared and regenerated nerve fibers, transection models are preferable. Lastly, the sometimes severe loss of function following contusion must be weighed against the potential benefits of using this type of lesion.

(2) A number of different types of incomplete spinal cord lesions are suitable for testing the effects of a treatment that promotes sprouting from spared axons and, perhaps, axon regeneration. This approach also is suitable for correlating axonal changes with specific behavioral improvements. An advantage of this preparation is that animals rapidly recover postural control following incomplete lesions, thereby allowing better assessment of arm and hand functions. Other key advantages of incomplete lesions in studying motor functions are as follows: (i) critical physiological functions such as bladder and bowel functions are preserved; (ii) less animal handling is needed because of partly spared postural and locomotor capabilities; and (iii) permanent, but modest impairments in both fine motor control and locomotion allow one to readily assess the potential of use-dependent mechanisms to enhance

recovery [22,31,32]. Surgically incomplete spinal cord transections provide an avenue for investigating the mechanisms of recovery that can be attributed to regeneration of specific ascending or descending tracts or to intra-spinal pathways. Mechanistic understanding of therapy-mediated motor recovery could be critical to the selection of a SCI population for a clinical trial, i.e., individuals with a complete lesion would not benefit from intervention-enhanced sprouting of spared fibres whereas such neural responses could promote significant improvements following incomplete injuries. Incomplete spinal cord transection models have some significant advantages over contusion models and retain sufficient relevance for the human condition to be a model of choice for testing the efficacy of many therapies. However, it could become important to test the effectiveness of some therapeutic interventions following incomplete contusion injuries if fundamental differences in the responses of the spinal cord to surgical and contused injuries are identified.

(3) Very significant insight into the mechanisms of neural plasticity which underlie recovery of posture and locomotion has been learned using the complete spinal cord transection model of mice, rats and cats. Experiments to date suggest that the mechanisms of recovery from complete and incomplete spinal cord injuries may be fundamentally different. Whereas true axonal regeneration may be the likely mechanism underlying functional recovery after a complete injury, local as well as supraspinal plasticity of intact fibres and their reorganisation may be the main factor after an incomplete injury [33]. Therefore, an anatomically complete transection injury could be another approach if the proposed therapy has been demonstrated to unequivocally induce true axonal regeneration in rodents. Nevertheless, the dramatic consequences of an anatomically complete transection injury on the general health of non-human primates, the associated psychological trauma for the animal, and the labor-intensive and skilled daily care required to maintain an acceptable state of health must be carefully balanced with the potential benefit for humans. Furthermore, the possibility of obtaining the information deemed to be most critical using rodents or other non-primate models must have been examined thoroughly prior to consideration of using this model in nonhuman primates.

C. Cervical and thoracic lesions present useful SCI models for translational studies in non-human primates

The level of an experimental spinal cord lesion, i.e., cervical, thoracic or sacral is an important decision. About half of the SCI in humans are at the cervical level and about half are at the thoracic or lower level. Experimental lesions at the cervical level allow one to study recovery of manual dexterity of the hand. This paradigm affords the most detailed assessment of the recovery of fine motor control and could be a major advantage of using non-human primates in the translation of a therapy to humans. Even limited sprouting or regeneration in the cervical spinal cord associated with recovery of some aspects of the fine motor function can be extremely beneficial to humans. Injuries of the non-human primate thoracic spinal cord allow the testing of recovery of locomotor and postural activities of the lower limbs as well as grasping of objects with the foot[15]. Thoracic injuries present a different type

of challenge from cervical lesions in that the descending and ascending tracts must form new connections directly and/or indirectly over much longer distances to become functional.

IV. Need for a comprehensive rehabilitative program for non-human primates concurrently administered with a neural regeneration-inducing intervention

Rehabilitation programs are an integral part of the care of human patients following SCI. When considered as a pre-clinical trial, regenerative interventions for the injured non-human primate spinal cord should be combined with a carefully controlled rehabilitation program since most interventions in humans will be accompanied by extensive rehabilitation. This scenario may be particularly important for regaining and maintaining precise motor control of the hand and digits. Critical variables related to activity need to be collected to define the interaction between use-dependent factors and the plasticity that can occur due to a regenerative intervention [28]. Indeed rehabilitation could drive the neural plasticity in a useful direction, while concomitant intervention-mediated effects could be manifested poorly without training. Theoretically, training improves the function of existing circuitries that can mediate coordinated movements. Nevertheless, we need to determine the optimal dosage and time point to start the regenerative intervention and rehabilitation in primates. There is much to be gained from the study of both fine motor control and locomotion in combination with neural regenerative and use-dependent factors in terms of translating SCI studies from non-human primates to humans. Since the recovery of function is a product of neurological and use-dependent factors, non-human primates are a superior human surrogate for the examination of the interactions of cognitive and motivational factors associated with physical therapy and other neurobiological interventions.

Summary

The non-human primate model provides unique advantages over rodents and other non-primate models for testing and understanding the safety and efficacy of reparative interventions to promote functional recovery following SCI in humans. The non-human primate provides an opportunity to examine, simultaneously and comprehensively, the effects of a regeneration-inducing intervention on multiple variables such as fine motor control of the arm and hand, as well as posture and locomotion (bipedal and quadrupedal), and autonomic control, e.g. bladder, bowel control and other autonomic functions. It is important to capitalize on these unique advantages in efforts to identify new ways to regain control of sensorimotor function of distal upper limb segments-digits. Furthermore, rehabilitative therapies involving skilled motor tasks and bipedal locomotion can be administrated in a prescribed and controlled way in non-human primates. Finally, the high risk of a hazard and/or malfunction that could accompany an invasive therapy in human subjects underscores the advantages of understanding the safety factors in the non-human primate before implementing an intervention on humans.

The pathway for developing the most effective and efficient route for delivering a novel intervention to the greatest number of patients would likely include experiments using non-human primates. Nevertheless, most of the fundamental work on neurological diseases, including SCI, can and should continue to be performed in rodents as well as other animals. Non-human primates should be used to test invasive neural interventions that are successful in non-primate species and, as a consequence, have a more reasonable potential for success in humans. Such studies can help to 1) pinpoint specific costs and benefits; 2) identify the mechanisms of recovery of function; and 3) elevate our confidence in the level of efficacy and safety of an intervention before considering human clinical trials. Given the common elements underlying a range of neuromotor disorders, the scope of the potential for capitalizing on the translational studies in non-human primates designed to augment motor recovery following SCI extends well beyond this specific injury.

References

1. Anderson, K.D. Targeting recovery: priorities of the spinal cord-injured population. *J Neurotrauma* 21, 1371-83 (2004).
2. Ackery, A., Tator, C. & Krassioukov, A. A global perspective on spinal cord injury epidemiology. *J Neurotrauma* 21, 1355-70 (2004).
3. Fawcett, J. Repair of spinal cord injuries: where are we, where are we going? *Spinal Cord* 40, 615-23 (2002).
4. Schwab, M.E. Nogo and axon regeneration. *Curr Opin Neurobiol* 14, 118-24 (2004).
5. Silver, J. & Miller, J.H. Regeneration beyond the glial scar. *Nat Rev Neurosci* 5, 146-156 (2004).
6. Thuret, S., Moon, L.D. & Gage, F.H. Therapeutic interventions after spinal cord injury. *Nat Rev Neurosci* 7, 628-643 (2006).
7. Tuszynski, M.H., Grill, R., Jones, L.L., McKay, H.M. & Blesch, A. Spontaneous and augmented growth of axons in the primate spinal cord: effects of local injury and nerve growth factor-secreting cell grafts. *J Comp Neurol* 449, 88-101 (2002).
8. Lemon, R.N., Kirkwood, P.A., Maier, M.A., Nakajima, K. & Nathan, P. Direct and indirect pathways for corticospinal control of upper limb motoneurons in the primate. *Prog Brain Res* 143, 263-79 (2004).
9. Rouiller, E.M., Moret, V., Tanne, J. & Boussaoud, D. Evidence for direct connections between the hand region of the supplementary motor area and cervical motoneurons in the macaque monkey. *Eur J Neurosci* 8, 1055-9 (1996).
10. Kuypers, H.G. The Descending Pathways to the Spinal Cord, Their Anatomy and Function. *Prog Brain Res* 11, 178-202 (1964).
11. Lemon, R.N. & Griffiths, J. Comparing the function of the corticospinal system in different species: organizational differences for motor specialization? *Muscle Nerve* 32, 261-79 (2005).
12. Bortoff, G.A. & Strick, P.L. Corticospinal terminations in two new-world primates: further evidence that corticomotoneuronal connections provide part of the neural substrate for manual dexterity. *J Neurosci* 13, 5105-18 (1993).
13. Lawrence, D.G. & Kuypers, H.G. The functional organization of the motor system in the monkey. I. The effects of bilateral pyramidal lesions. *Brain* 91, 1-14 (1968).
14. Freund, P. et al. Nogo-A-specific antibody treatment enhances sprouting and functional recovery after cervical lesion in adult primates. *Nat Med* 12, 790-792 (2006).
15. Courtine, G. et al. Performance of locomotion and foot grasping following a unilateral thoracic corticospinal tract lesion in monkeys (Macaca mulatta). *Brain* 128, 2338-58 (2005).
16. Nathan, P.W. Effects on movement of surgical incisions into the human spinal cord. *Brain* 117 (Pt 2), 337-46 (1994).
17. Muir, G.D. & Whishaw, I.Q. Complete locomotor recovery following corticospinal tract lesions: measurement of ground reaction forces during overground locomotion in rats. *Behav Brain Res* 103, 45-53 (1999).

18. Schmidlin, E., Wannier, T., Bloch, J. & Rouiller, E.M. Progressive plastic changes in the hand representation of the primary motor cortex parallel incomplete recovery from a unilateral section of the corticospinal tract at cervical level in monkeys. *Brain Res* 1017, 172-83 (2004).
19. Whishaw, I.Q., Gorny, B. & Sarna, J. Paw and limb use in skilled and spontaneous reaching after pyramidal tract, red nucleus and combined lesions in the rat: behavioral and anatomical dissociations. *Behav Brain Res* 93, 167-83 (1998).
20. Dancause, N. et al. Extensive rewiring after brain injury. *J Neurosci* 25, 10167-79 (2005).
21. Wannier, T., Schmidlin, E., Bloch, J. & Rouiller, E.M. A unilateral section of the corticospinal tract at cervical level in primate does not lead to measurable cell loss in motor cortex. *J Neurotrauma* 22, 703-17 (2005).
22. Schmidlin, E. et al. Reduction of the hand representation in the ipsilateral primary motor cortex following unilateral section of the corticospinal tract at cervical level in monkeys. *BMC Neurosci* 6, 56 (2005).
23. Belci, M., Catley, M., Husain, M., Frankel, H.L. & Davey, N.J. Magnetic brain stimulation can improve clinical outcome in incomplete spinal cord injured patients. *Spinal Cord* 42, 417-9 (2004).
24. Darian-Smith, C. & Ciferri, M.M. Loss and recovery of voluntary hand movements in the macaque following a cervical dorsal rhizotomy. *J Comp Neurol* 491, 27-45 (2005).
25. Courtine, G. et al. Effects of a unilateral corticospinal tract (CST) lesion or a complete cervical hemisection (HS) on locomotor performance in a primate. Program No. 654.7 (Soc Neurosci Abstr., Washington, DC, 2004).
26. Nakajima, K. et al. Biomechanical constraints in hindlimb joints during the quadrupedal versus bipedal locomotion of M. fuscata. *Prog Brain Res* 143, 183-90 (2004).
27. Edgerton, V.R., Tillakaratne, N.J., Bigbee, A.J., de Leon, R.D. & Roy, R.R. Plasticity of the spinal neural circuitry after injury. *Annu Rev Neurosci* 27, 145-67 (2004).
28. Friel, K.M., Heddings, A.A. & Nudo, R.J. Effects of postlesion experience on behavioral recovery and neurophysiologic reorganization after cortical injury in primates. *Neurorehabil Neural Repair* 14, 187-98 (2000).
29. Hofstetter, C.P. et al. Allodynia limits the usefulness of intraspinal neural stem grafts; directed differentiation improves outcome. *Nat Neurosci* 8, 346-353 (2005).
30. Medical Research Council & Wellcome Trust Primates in Medical Research. (London, 2006).
31. Yang, H. et al. BDNF and NT3 gene delivry in models of primate spinal cord injury (SCI). Program No. 106.14 (Society for Neuroscience, Washington, DC, 2004).
32. Wannier, T. et al. Behavioral recovery from cervical hemisection in monkeys is enhanced by anti-nogo-A treatment. Program No. 103.2 (Soc Neurosci Abstr., Washington, DC, 2005).
33. Fouad, K., Klusman, I. & Schwab, M.E. Regenerating corticospinal fibers in the Marmoset (Callitrix jacchus) after spinal cord lesion and treatment with the anti-Nogo-A antibody IN-1. *Eur J Neurosci* 20, 2479-2482 (2004).