Translational Challenges of Rat Models of Upper Extremity Dysfunction After Spinal Cord Injury

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There are approximately 17,500 new spinal cord injury (SCI) cases each year in the United States, with the majority of cases resulting from a traumatic injury. Damage to the spinal cord causes either temporary or permanent changes in sensorimotor function. Given that the majority of human SCIs occur in the cervical spinal level, the experimental animal models of forelimb dysfunction play a large role in the ability to translate basic science research to clinical application. However, the variation in the design of clinical and basic science studies of forelimb/upper extremity (UE) function prevents the ease of translation. This review provides an overview of experimental models of forelimb dysfunction used in SCI research with special emphasis on the rat model of SCI. The anatomical location and types of experimental cervical lesions, functional assessments, and rehabilitation strategies used in the basic science laboratory are reviewed. Finally, we discuss the challenges of translating animal models of forelimb dysfunction to the clinical SCI human population. **Key words:** *animal models, forelimb, functional recovery, upper extremity*

According to the most recent annual report from the National Spinal Cord Injury Statistical Center, 54.2% of persons with a spinal cord injury (SCI) have cervical lesions at discharge.[1] Upper extremity (UE) recovery is of high clinical value because it is the primary factor of functional independence.[2] The use of the UE is critical in completing basic activities of daily living (ADLs) such as self-feeding, dressing, bathing, and toileting.[3] Therefore, the ability to preserve function in the cervical spinal cord can result in a substantial improvement in an individual's quality of life (QOL) and independence. Experimental incomplete contusion and compression models of clinical SCI have been used for more than a century to understand the histopathological and functional consequences of injury.[4] Over the last several decades, the function of the forelimb, the "upper extremity" of most species used in experimental SCI, has been queried by a variety of behavioral assessments that measure the residual reflexive, motor and somatosensory function. While each assessment measures a different skill of the forelimb, the collective data suggest that forelimb deficits following experimental cervical SCI mimic quite closely the UE deficits seen in clinical SCI. Cellular, molecular, and rehabilitative strategies to improve function have been widely tested in experimental cervical SCI models,[5-9] yet the ability to translate information from basic science findings to the clinical SCI community is limited. This challenge is not unique to the SCI community.

Silberberg showed that many preclinical studies do not provide the needed methodological information related to experimental design, conduct, and data analysis[10] and suggests that false positive reporting and inability to recapitulate results across species and/or laboratories is due to a lack of randomization reports, allocation concealment or blind assessment of outcomes, and underpowered studies. Further, Reier et al suggest that improvements of preclinical experimental design that address the lack of consistencies across

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laboratories in addition to unavoidable differences between human and experimental SCI will generate more effective, translatable therapies.[11] Therefore, this review will give an overview of experimental models of SCI-induced forelimb motor and sensory dysfunction and discuss the translational applicability and challenges of translation from experimental models to the clinic.

Review of Experimental Models of SCI

Several mammalian species, including non-human primate, cat, dog, pig, mice, and rats, have been used experimentally to model clinical SCI.[12-23] The direct cortical projections to spinal motor neurons correlate with the emergence of precision grip between the thumb and index finger, something only seen in some primates.[14,24,25] Thus, the use of non-human primates to identify the underlying sensorimotor control of the precision grip and to develop effective interventions to recover this important UE function is likely essential. However, there are few research labs that are equipped to carry out studies in non-human primates. Non-human primate studies are costly, and there are many delicate ethical concerns surrounding non-human primate use.[26-28]

Historically, cats were most frequently used as experimental SCI subjects as their spinal cord is of similar diameter and length to the human. Even though cats are typically thought to have poor digit dexterity, they incorporate skilled forelimb and paw movements when hunting prey. In the 1980s, tract-specific lesions were conducted in cats to determine the brain regions and spinal pathways that controlled forelimb and paw function.[29-33] Gorska and Sybirska[34] developed a functional assessment of forelimb and paw function in the cat following spinal cord lesions. Cats were trained to retrieve a small food reward from a narrow tube and bring it to their mouth, often using their claws and footpads to hold and grasp food. After the anatomical underpinnings were determined, the field moved toward using animals that had dexterous movements similar to humans.

Rodents are the most frequently used animals in experimental SCI, likely for availability, relative ease of care, and financial and ethical reasons. Although the finesse in digital control is far less developed in rodents when compared to humans and non-human primates,[14] there are many functional assessments of the forelimb in the rodent models of SCI (see **Table 1**).[20,35-50] Mouse models of SCI permit the use of genetically engineered models allowing for the evaluation of molecular and cellular changes following SCI, and they are beginning to be used for understanding forelimb movement and dexterous behavior.[51] However, mice display a wound healing process that is different following SCI when compared to other species including rats and humans.[52] In the mouse, there is no cavitation at the lesion site but rather the lesion is fill in with connective tissue.[52] Given the uniqueness in the healing process in addition to the limited data available using murine models of UE dysfunction, they will not be further discussed in this review. The remainder of this review will focus on the comparison between rat and human SCI.

Anatomical Location

The anatomical level of the spinal cord lesion (eg, C1-T1) is one of the many factors that contribute to functional recovery. The myotomes and dermatomes innervated by the cervical spinal

Table 1. Functional assessments used in experimental rat cervical spinal cord injury

Motor assessments	Sensory assessments	Sensorimotor assessments
Overground locomotion[35-37]	Paw tactile allodynia (von Frey)[44]	Horizontal ladder[20]
CatWalk[38]	Paw thermal hyperalgesia[44]	Tape removal test[46]
Single pellet retrieval[39,40]	Operant tactile avoidance[45]	Cereal manipulation task[47,48]
Staircase pellet retrieval[41]		Pasta test[49,50]
Paw use preference[42]		Grooming[20]
Grip strength[43,66]		

cord are conserved in humans and rats (**Table 2** and **Figure 1**). Lesions in the upper cervical spinal cord elicit deficits in proximal musculature of the UE or forelimb that are important for limb lift (eg, biceps and deltoids) while lower cervical injuries are more likely to affect limb extension (eg, triceps) as well as digit function across mammalian species (**Table 2**). [53] Somatosensory deficits are not as simple to dissect, as somatosensory and pain information from dermatomes are received at every level of the spinal cord via primary afferent fibers from the dorsal root ganglia and may reflect direct damage to the afferent input itself or damage to the projection neurons in the dorsal horn of the spinal cord. Further complications in assessment may arise from the fact that the pain (spinothalamic tract) and light touch (dorsal column-medial lemniscus) pathways ascend in opposite spinal cord hemispheres (**Figure 2**).

Table 2. Spinal cord level associated with forelimb and upper extremity muscles in the rat and human

	Spinal level	
Key myotome	**Rat**	**Human**
Elbow flexion—biceps and brachialis	C4-C5	C5
Wrist extension—extensor carpi radialis longus and brevis	C6-T1	C6
Elbow extension—triceps	C7-C8	C7
Middle finger flexion—flexor digitorum profundus	C8-T1	C8
Little finger abduction—abductor digiti minimi, interossei	C8-T1	T1

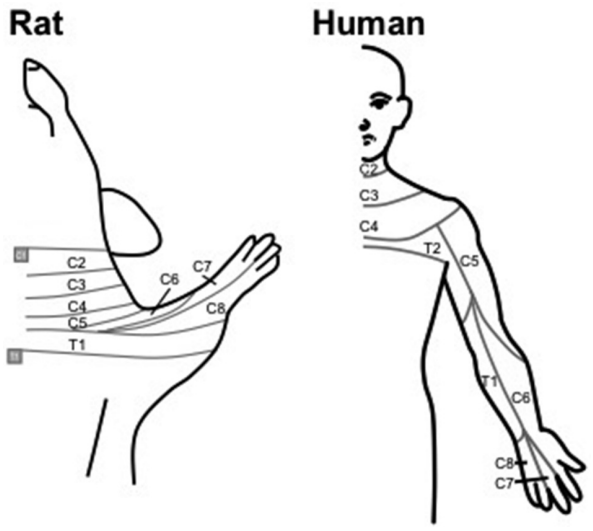

Figure 1. Schematic of sensory dermatomes in rat and human. Forelimb and upper extremity dermatomes are conserved in rat and human. Transmission of somatosensory information from the periphery is transmitted to similar spinal cord levels in rats and humans.

The dorso-ventral and mediolateral location of the injury within the cervical spinal cord will also dictate functional recovery. For the most part, the locations of motor and sensory white matter tracts are similar in rats and humans.[14,54] **Figure 2** shows a schematic comparison of the human and rat cervical spinal cord with the ascending and descending spinal cord tracts identified. Notably, unlike humans, rats have a prominent dorsal corticospinal tract (CST) that sits at the base of the dorsal columns with a small lateral and ventral CST. Additionally, the relative size of the rubrospinal tract (RST) is more prominent in rats compared to in humans, In rats, the RST extends the full length of the spinal cord,[55] and it is well established that it is the primary tract responsible for voluntary forelimb and hindlimb motor function in rats. Importantly, the CST is not required for noncomplex motor movements in rats and other lower mammals.[56,57] However, in humans, the control of voluntary motor function is regulated primarily by the CST and not the RST. [58] In the human spinal cord, the RST terminates in the cervical segments of the spinal cord and only is involved in UE flexion, [55,59,60] whereas the CST extends the length of the spinal cord. Lemon et al found that the importance of this tract in terms of motor control correlates with developmental changes that occur during primate evolution. [24] These differences in tracts that drive motor control of the UE and forelimb are important to recognize, as an interruption of the cortical projections to the spinal cord via the CST in humans results in a marked deficit in fine motor function of the hand and feet and only modest forelimb deficits in rodents.[13]

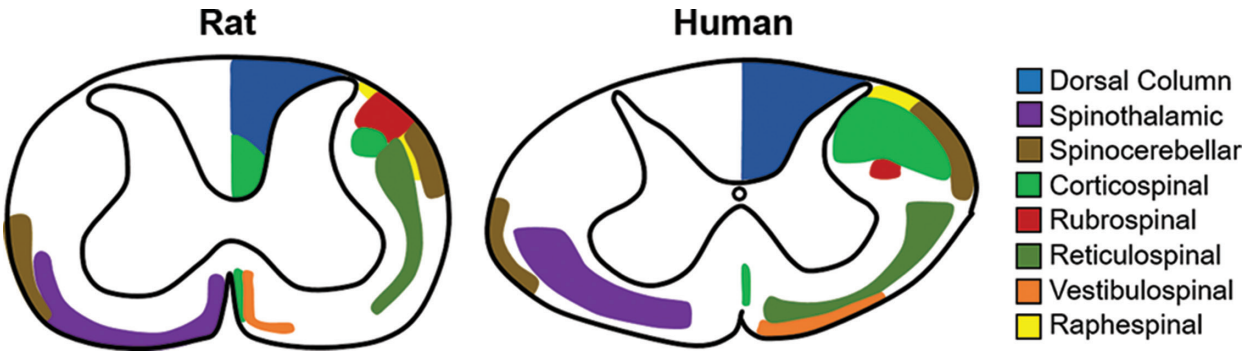

Figure 2. Schematic of spinal cord tracts in the rat and human that would directly or indirectly innervate the left forelimb or upper extremity, respectively. Note that dorsal spinocerebellar tract ascends in the ipsilateral white matter, while the ventral spinocerebellar tract ascends in the contralateral white matter of both species. The locations of the corticospinal tracts are markedly different between rats and humans. Both species have a lateral and ventral corticospinal tract, but the rat also has a dorsal corticospinal tract at the base of the dorsal columns.

Types of Experimental Cervical SCI

SCI in the cervical cord can be generated experimentally using surgical, chemical, contusive, and compressive approaches. Surgical approaches use a sharp blade to cut specific regions of the spinal cord, damaging a small portion of grey matter at the site of the incision, with the predominant injury being axotomy of ascending and descending white matter tracts.[61] Common surgical lesions are a dorsal column section; a dorsal hemisection, which sections the dorsal columns, dorsal horn, and dorsolateral white matter; and a lateral hemisection, which disrupts all white and grey matter of one spinal cord hemisphere. The most common lesion for the assessment of UE function is the contusion, specifically at the C4-C5 level.[5,9,20] Contusion and compression of the spinal cord cause widespread damage to grey matter at the injury epicenter and disruption of multiple ascending and descending tracts that mitigate motor and sensory function. It is important to note that contusive SCI models in the cervical spinal cord are often lateralized, thereby limiting the lesion to one hemisphere of the spinal cord to limit respiratory complications and essentially ensure better overall health status of the animal. Animals with unilateral cervical SCI are able to locomote around their home cage to gain access to food and water and are able to void their bladder and bowels independently, and there is no need for acute artificial ventilation. The histopathology shows a cystic, fluid-filled cavity that is often limited to or is predominantly on one side of the spinal cord that is surrounded by a rim of spared white matter. Bilateral cervical contusions on the other hand often leave the animal with far less functional independence. Typically, animals that have sustained a bilateral cervical contusion will be kept on a ventilator during the SCI itself as well as in acute recovery; their locomotor ability and ability to eat and drink are compromised during the subacute period post SCI. These deficits make it inherently difficult to initiate a rehabilitative intervention within the first few weeks post SCI. The severity of contusive cervical injury can be controlled by the force applied to the spinal cord. While contusive SCI offers the opportunity to assess recovery of function and therapeutic interventions in a more clinically relevant scenario, it may not be the best to assess the specific contributions of a singular tract on motor or sensory control and/or recovery as a surgical or chemical lesion of specific white matter tracts would.

Functional Assessments of Forelimb Dysfunction

In the research lab, the measurement of UE and forelimb function in rodents with a cervical SCI uses behavioral assessments that assess

reflexive, motor, and sensory function (**Table 1**). Like humans and non-human primates, rats use their forelimbs in feeding, grooming, and exploratory behaviors, and the actions of reaching and grasping objects is evolutionarily conserved across many mammalian species.[62] Development of functional assessments of similar behaviors like the reach-to-grasp movement across species may increase the likelihood of successful translation of therapeutic strategies from rodent models of UE dysfunction to the clinic. An important caveat is that, unlike humans, rodents are quadrupedal and require the use of their forelimbs to locomote, and assessment of this function of their forelimb, while seemingly less relevant to humans, should not be overlooked. Interlimb coordination is essential for normal locomotion in rats but not humans. SCI at the cervical level in both species damages long ascending and descending propriospinal neurons that connect locomotor central pattern generators in the cervical and lumbar enlargements.[63] Recovery of overground locomotion in animals may impact the ability to perform reaching and grasping tasks, as repetitive loading of the affected forelimb will reduce muscle atrophy and strengthen limb musculature.[64,65] There are many functional assessments of locomotor recovery ranging from gross locomotor ability to detailed gait metrics and assessments of interlimb coordination that could be utilized as an additional measure of forelimb recovery (**Table 1**). Unlike a grasping task, locomotion does not require digit dexterity or grip strength. However, the rat's forelimb must generate relatively large, coordinated movements of the shoulder, elbow, and ankle joints during locomotion, suggesting that their locomotor performance could be an indicator of their ability to extend and reach for an object.

Rehabilitative Strategies

Although there are many similar features in rehabilitation protocols within the experimental and clinical settings, there are also many differences. As in humans, rehabilitation following experimental SCI is also a widely used intervention to enhance functional recovery. Experimental rehabilitation or exercise paradigms that improve forelimb function in rats include aerobic[6,9] (treadmill training, forced or voluntary running wheel), strength/resistance training[66] (isometric lever pull task), and task-specific[5,61] (pellet retrieval task) paradigms. Modeled after clinical SCI rehabilitation, these interventions are repeated three to five times per week at equivalent intensities used in the clinical setting. The time of initiation post SCI varies across experiments depending upon the scientific question. As seen in the clinic, basic science researchers are still unclear in regard to the "best practice" given the heterogeneity of the SCI models that exist. Currently there are no standard protocols that indicate the optimal training paradigms to achieve robust gains in function. Whether strength training, task-specific reach-to-grasp training, or a combination of both is most effective may depend on the individual subject. Further, the timing of rehabilitative intervention and the frequency and intensities to promote maximal functional recovery are not currently known. Dose-response curves are not often reported for rehabilitative training in experimental SCI, and they focus more on a single intensity or type of training.

There are also a number of differences in rehabilitation protocols between experimental and clinical SCI populations that include but are not limited to the following:

- *Availability*. The animal is always available, whereas a human patient can be delayed for many reasons.
- *Motivation*. As most animal models use food restriction and/or reward to motivate performance on the rehabilitative task, the emotional state of a person following SCI can present a challenge that can interfere with the success of rehabilitation.
- *Multiple Organ Dysfunction.* As most human SCIs are traumatic in nature, the possibility of injuries to other organs and systems exist. These include insult to the brain, lungs, cardiovascular system, spleen, urinary system, skeletal muscle, bone, soft tissue, and skin.[67] While these dysfunctions occur in the animal model, for example, urinary tract infections and pressure sores, those caused by the traumatic insult such as brain injury and lung punctures are not commonly studied. Basic scientists are just beginning to develop experimental models of polytrauma that include

both SCI and traumatic brain injury in an effort to better understand the clinical situation. This offers a new avenue to explore mechanisms to improve recovery of function when cognitive centers in the brain are also impacted.[68]

- *Equipment and Facilities.* As mentioned above, there are a number of rehabilitation paradigms used in preclinical research, all of which involve different types of equipment. While this equipment can be costly, the cost of rehabilitation equipment in the clinic is more expensive. In addition, there are a limited number of facilities that specialize in SCI rehabilitation that have both the needed equipment and trained personnel.
- *Cognition and Communication*. A human can understand and comprehend what the therapist is instructing them to do and make the needed adjustments. However, the rat is undergoing a training session for a reward, often food. In order for adjustments to be made, the researcher may need to devise a different set up to avoid the unwanted movement.
- *Psychological.* We can not fully determine the psychological state of a rat, and there are factors other than the direct psychological state of a person such as family dynamics, caregiver burden, housing (return home or nursing home), and availability of rehabilitation based on insurance that do not exist in the preclinical model.

Challenges of Translation

Although experimental animal models provide a substrate for researchers to understand the efficacy of various interventions, there are many challenges involved in the translation of these findings to human SCI. One main issue with translation from experimental to clinical SCI is the homogeneity of the injury type, in addition to lesion severity and location. Most animal studies in the cervical spinal cord are designed to elicit a standard and lateralized spinal cord lesion. This reduces respiratory complications and allows for a large number of subjects with very similar lesions to receive the same intervention. Thus, the likelihood that these experimental animal subjects would respond similarly to an intervention is relatively high. This relatively uniform experimental cohort is not seen in the human clinical trials given that human SCI is notoriously heterogeneous.

Recovery of a motor function is preferable over the use of compensatory strategies that often result following an SCI. Although the overall goal of functional recovery in animal models of SCI is also to limit the amount of compensation, preventing compensatory movements is a challenging task. As most experimental cervical SCIs are unilateral, preventing the compensatory use of the uninjured forelimb is often difficult. Even constraining the uninjured (contralesional) forelimb in a similar fashion to constraint-induced movement therapy can be modestly effective. Likewise, most animals have an instinct to remove anything that prevents their ability to move freely, resulting in the animals focusing on becoming "free" rather than the rehabilitative task at hand. The inability to "reason" and verbally communicate with the animals as one would with a human further complicates the rehabilitative situation. In order to increase motivation to perform the given task, animals are often food restricted and given a food reward on correct completion of the rehabilitative task [65,69] Alternatively, negative reinforcement techniques such as electric tail or foot shock may be utilized to punish the animals for failure to complete a task correctly. [69] Both positive and negative reinforcement strategies are used to motivate SCI animals to perform during behavioral assessments and administration of rehabilitative therapies.[61,66] However, negative reinforcement techniques are rarely used, as it is unclear whether neural circuits that transmit noxious information to the brain are unaltered after SCI. A satiated rat will rarely reach for the treat in a single pellet retrieval task or pull a lever for a food reward. Either way, with negative or positive reward, experimenters are assessing motivated and not voluntary behaviors of the rat and this represents an inherent difference between rodent and human rehabilitation. There are some examples where rats are not food restricted and are allowed to complete tasks based on their own volition. Reaching and grasping tasks that are set in the home cage[40] and the forepaw dexterity assessment (Irvine, Beatties, and Breshanhan cereal manipulation task[47]) rely on the animal's willingness to pull the lever or eat the sugared

cereal rather than using positive reinforcement techniques. While a compensatory strategy is not the first thing the clinical rehabilitation team plans to use, the severity of the injury and the degree of functional recovery oftentimes lends itself to use of adaptive equipment. These adaptive strategies are rarely implemented in experimental animal studies.[70]

The unexpected nature of an SCI event, the robust heterogeneity in human SCI, injury severity, and other elements that are out of the control of the treatment team, such as wound healing due to skin breakdown, regulation of various autonomic complications (hypotension, autonomic dysreflexia, temperature regulation, etc), and the need for psychological and emotional support, may affect the application and/or administration of therapeutic interventions. Even if delaying a treatment strategy is the goal for the SCI individual, the time of an intervention (cell transplant, drug delivery, etc), the start of rehabilitation often occurs later post SCI in the human population than in experimental animal models. The controlled nature of the animal research allows for all aspects of the study, including the timing of interventions and behavioral assessments, to be well planned prior to the SCI, thereby removing variability from the study design.

Clinical and experimental basic scientists are not limited by the constraints of insurance companies and can employ assessments that require a large amount of training, data collection, and analysis. For example, while not a translatable assessment, the Graded Redefined Assessment of Strength, Sensibility and Prehension (GRASSP) test[2] was developed to distinguish between functional and neurological changes in a person with incomplete tetraplegia. This assessment involves a variety of qualitative and quantitative components that are extremely valuable in assessing functional improvements caused by interventions. However, imposed time limits to rehabilitative therapy for SCI patients often prohibits the use of these detailed assessments in clinical practice.

If the end goal of conducting preclinical studies is to translate experimental findings into clinical practice, careful considerations must be given to the study design in the animal models. Fouad et al identified five main areas to consider when conducting functional testing in animal models of SCI.[71] These areas included lesion size and its correlation with degree of recovery, compensation and motor recovery, types of outcomes to score recovery, challenges of animal testing, and training versus testing. This information further solidifies other researcher's views on the importance of methodological design in preclinical animals models.[10,72] The relevance of quantifiable, statistical significance on a functional assessment and functional gain are not always synonymous. One possibility to improve agreement between the outcomes of experimental studies and clinical practice could be to implement assessments of forelimb and UE function of a binary (yes/no) nature to accommodate the clinical situation where therapy sessions are limited. When designing an assessment in an animal model of UE dysfunction that is intended to measure a similar function in human, it is important to keep in mind the goal of the measure and whether this test will be feasible to translate it to the clinic.

The overall goals of what basic scientists and clinicians aim to achieve are different. Animal studies are designed to answer a specific question, whether it is about the success of a cell transplant, nerve graft, pharmacological, or sensorimotor intervention. Even though part of the human SCI experience may involve rehabilitation and enrollment in a research study that aims to answer a specific question, the overall goal of the clinical management team is to restore as much independence as possible to an individual. Therefore, the main focus for someone in rehabilitation post SCI is to improve independence in completing ADLs. In particular, someone with a cervical SCI can increase their independence by being able to dress, cook, feed himself or herself, and perform their own bowel and bladder program. In the laboratory, investigators can measure grooming behavior in rodents; yet the test does not measure how clean the rat becomes but rather defines the rat's forelimb range of motion as determined by the completeness of the grooming motion.[18] Alignment of the goals of the two fields could be achieved by altering the question asked or by adding to the interpretation of existing rodent behavioral assessments to be consistent with clinical ADLs.

Conclusion

Research in the experimental models of SCI has evolved over the last three decades, but the ability to translate these findings into the human SCI population still remains a challenge. The differences among species anatomy, lesion models, and goals of functional assessment, to name a few, pose a set of unique and challenging situations that are not clear cut and easy to solve. For instance, using non-human primates to study recovery of UE extremity function following SCI would solve the problem of anatomy differences but this model is less favorable in most study designs because the study would be restricted to one of the few labs that is capable of this research in addition to the difficulty of obtaining the sample size needed. While attainable, both the basic science and clinical researchers in addition to the practicing clinicians have to commit to come together to close the gap between disciplines in order to provide meaningful advances for the SCI community.

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