Translational measures of behavioral function after spinal cord injury: A multivariate study of outcomes across species

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The past 20 years have seen significant progress in our understanding of the pathophysiology of spinal cord injury (SCI). As highlighted by the other presenters in this session, advances in our ability to measure behavioral changes in animal models have made a significant contribution toward this progress. In recent years a number of authors have developed new sensitive and reliable measures that provide insights into a range of functional states including locomotor function, forelimb function, autonomic function and sensory function after SCI. In addition, the rapid expansion of our understanding of the basic neuroscience of central nervous system trauma and plasticity has provided unprecedented opportunities to identify and target the mechanisms of injury with new therapeutics. Yet, to date, there have been few examples of new therapies that have made the translational leap from basic neuroscientific research to clinical application.

These translational difficulties may be due, in part, to the intrinsic complexity of SCI coupled with limitations of the analytical techniques that are commonly used to deal with SCI data. To understand the biological processes involved in SCI, researchers have developed animal models that mimic functional changes experienced by humans. However, the value of animal models depends, in part, on their ability to emulate the constellation of biological and functional changes observed in human SCI. Currently, there are many sensitive measures of behavioral function in animal models (Figure 1), however there is no unified strategy for integrating these functional measures to gain insights into the mechanisms underlying alteration and recovery of function following a SCI. One issue is the lack of universal standards for data acquisition, data formatting, and data sharing within the basic research community. This lack of standards reduces the replicability across laboratories and across species, and limits the translational potential of SCI findings. A second major problem is that quantitative integration of behavioral measures requires sophisticated statistical techniques that are not commonly used within the animal SCI literature.

In the prevailing approach, researchers attempt to understand the relationship between biology and functional behavior using simple correlation or stepwise regression: one functional measure is correlated with one biological measure. This process is then repeated many times in an attempt to assess how different functional measures relate to different biological outcomes. Researchers often apply a similar analytic strategy in an attempt to understand relationships between different functional measures: one measure is correlated with another, and this process is repeated for all comparisons of interest. However, there are major limitations with this type of analysis. First, this approach does not allow one to assess the degree of redundancy among different functional tests. Second, it is insensitive to complex, systematic changes that exist across several inter-related outcome measures. However, it is these multivariate changes that define the *syndrome* and are the target of therapeutic intervention (Figure 2).

Multivariate statistics such as principal components analysis provide a powerful alternative approach. This family of analytical techniques detects clusters of association among many different outcome measures while remaining sensitive to detailed information provided by each of the individual tests. In the context of SCI, such multivariate clusters can help identify the relevant groups of outcomes for a particular question of interest (e.g., neuroprotection vs. plasticity) or the

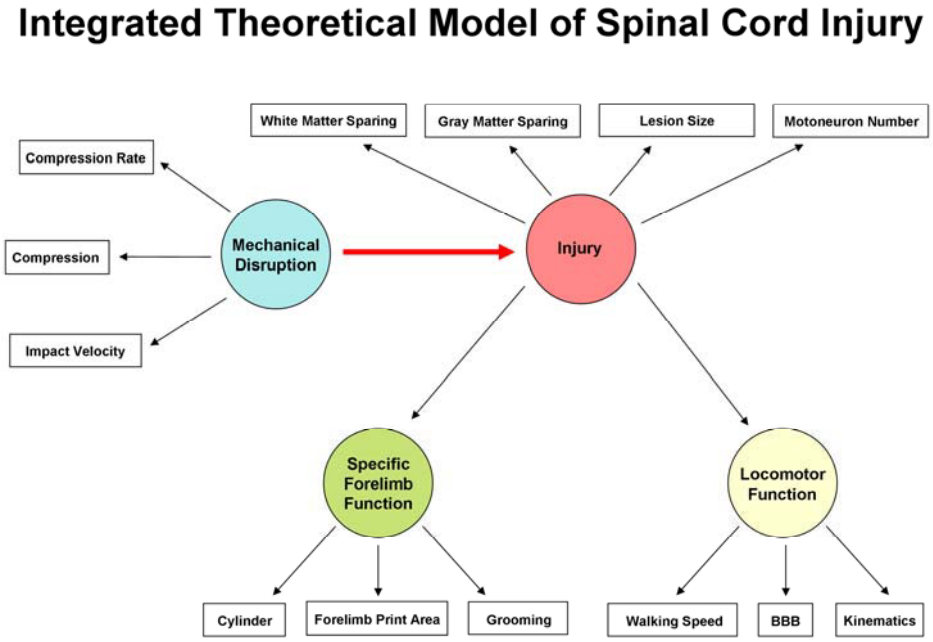

Figure 1. *Example of a simple integration scheme for a "typical" SCI experimental dataset. It is common for researchers to collect information within the same experimental subjects on the basic biomechanics of the injury, histological changes, and functional changes as measured on behavioral outcomes. However, these rich datasets are typically deconstructed with univariate statistical methods (e.g., correlation; ANOVA) which ignore information about the overlap among measures.*

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outcomes that span several functional elements (Figure 2).

Here we illustrate the strength of this approach by applying multivariate techniques to identify, compare, and contrast syndrome-level features of cervical SCI in rodents and primates. The results identify a subset of outcomes that provide a unique window into the critical multivariate features of SCI in both rodents and primates. For example, within rat datasets, contusion injury affected a subset of behavioral measures (grooming, paw preference, print area an automated walkway) which had significant multivariate overlap with histology, forming a single multivariate factor (Factor 1). Other behavioral tests clustered together into different factors representing stability, locomotion, and horizontal ladder performance. These other factors were unrelated to tissue sparing, suggesting that these outcomes tap into subtle biological changes undetected by standard histology. As validation, we then tested the effect of graded cervical spinal cord injury using these multivariate features as the outcomes. The severity of SCI only had a significant effect on Factor 1, indicating that this cluster of measures is most sensitive to injury processes and should be used to evaluate therapeutic interventions that target these processes. Applying a similar approach to primate data, we were able to identify therapeutic effects that related to tissue damage, but were unrelated to functional recovery, suggesting that important therapeutic targets have yet to be discovered. Comparison of primate and rodent data revealed that a subset of tests showed temporal similarities across species in terms of behavioral losses and recovery. This suggests that SCI may engage analogous mechanisms in different species, and that it may be possible to select behavioral outcomes that are sensitive to translational changes. Further work is required to determine which measures represent translational outcomes and which reflect species-specific and model-specific changes. In the future, by targeting therapeutic interventions to translational multivariate features, SCI research may have improved success at discovering new therapeutic interventions that can be applied in a clinical setting.

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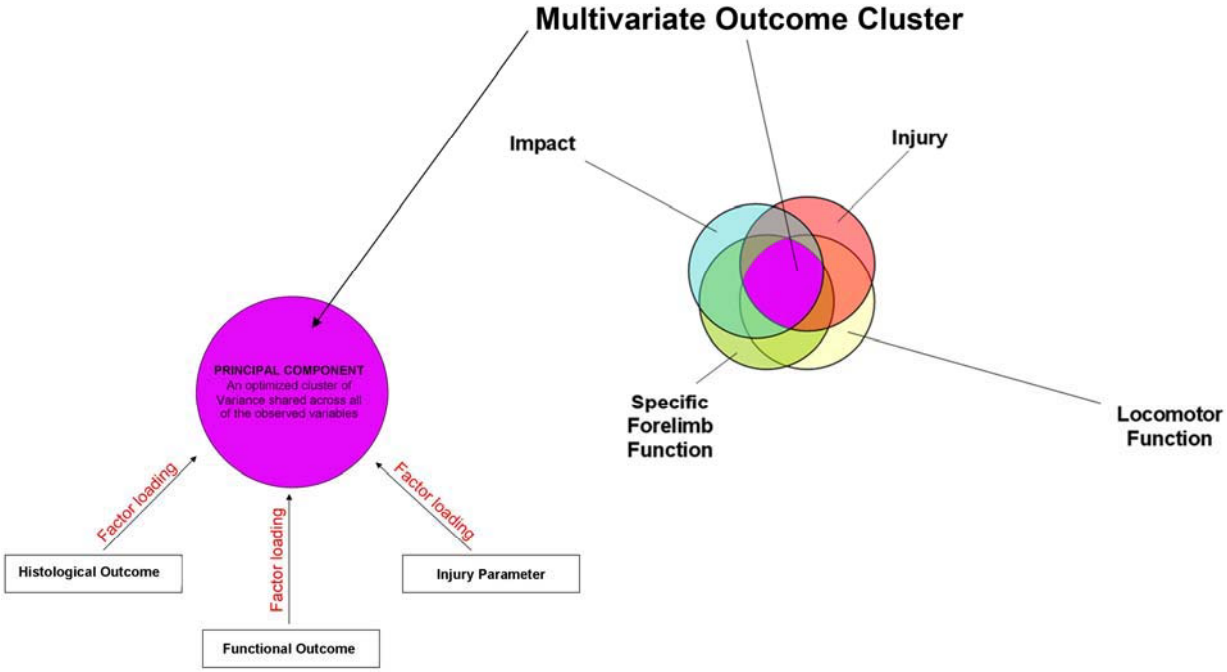

Figure 2. *Schematic illustration of the application of a multivariate method (in this case, principal components analysis) to identify clusters of outcomes within a typical SCI dataset. It is possible for clusters to reflect specific elements of SCI (e.g., impact biomechanics, tissue injury, or functional states) or overlap among all of the elements.*