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Review Article

Rodent spinal cord injury models for studies of axon regeneration

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ABSTRACT

For over a century, axon regeneration has been considered the Holy Grail for spinal cord injury (SCI) repair. Although there are other factors that could contribute to improving function, restoring the long motor and sensory tracts that are interrupted by SCI has the greatest potential for actually reversing paralysis, restoring the brain's control of autonomic functions mediated by sympathetic and parasympathetic circuits of the spinal cord and restoring sensation. Accordingly and in keeping with the overall theme of this special issue, this review focuses narrowly on rodent SCI models for studies of axon regeneration.

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1. Introduction

For over a century, axon regeneration has been considered the Holy Grail for spinal cord injury (SCI) repair. Although there are other factors that could contribute to improving function, restoring the long motor and sensory tracts that are interrupted by SCI has the greatest potential for actually reversing paralysis, restoring the brain's control of autonomic functions mediated by sympathetic and parasympathetic circuits of the spinal cord and restoring sensation. Accordingly and in keeping with the overall theme of this special issue, this review focuses narrowly on rodent SCI models for studies of axon regeneration.

1.1. What does the term "axon regeneration" actually mean?

Unfortunately, there is still a great deal of inconsistency in the use of terminology to refer to axon growth following injury. Here, we follow the definitions in Tuszynski and Steward (2012). In the context of spinal cord injury, the term *axon regeneration* is reserved for growth of a cut axon with extension into or beyond the lesion. Regenerating axons may either extend through a lesion, through something that is implanted (peripheral nerve bridge, cell or tissue graft, or scaffold), or around the lesion through surviving white or gray matter. Regenerating axons may end abortively (functionally irrelevant), form ectopic connections (could be either beneficial or detrimental to function), or connect with normal targets (likely to restore function).

It is not straightforward to prove that axons are regenerated and not spared; criteria to identify regenerated axons are discussed elsewhere (Steward et al., 2003). As discussed further below, lesion models differ in the extent to which these criteria can be applied definitively.

There are other forms of regenerative axon growth following injury that differ from canonical regeneration, for example, *collateral sprouting* and *regenerative sprouting*. *Collateral sprouting* refers to growth by an axon that has *not* been directly damaged. An example is trans-midline growth of CST axons from one side after unilateral pyramidotomy. *Regenerative sprouting* refers to growth from a cut axon where the new growth arises from a part of the axon near the cut end rather than the cut end itself.

As noted previously (Tuszynski and Steward, 2012), terms for regenerative growth have been used inconsistently and imprecisely; also, anatomical studies are often not carried out in a way that makes it possible to actually determine which form of growth is occurring. Accordingly, the best approach for clarity is to describe the actual anatomical changes in short phrases, even if this is cumbersome.

Recently, however, there is growing interest in developing databases for meta-analyses. For these to be useful, it will be important for the field to adopt standardized terminology for different growth phenomena and use terms consistently. Accordingly, for indexing and database development, we propose that *axon regeneration*, *collateral sprouting* and *regenerative sprouting* be used as defined as above. Sprouting that arises from part of the axon that is distant from an injury could be a separate category. An example would be formation of new collaterals by CST axons at cervical levels after a distal injury at the thoracic level. If the form of growth is uncertain, we suggest the indexing term *axon growth of unspecified form*.

1.2. Considerations for injury models to study axon regeneration

The factors that make a good model for studies of axon regeneration are different than for studies of neuroprotection and recovery of function. For studies of neuroprotection and recovery, contusion injuries created by impactors have become the industry standard, but there are caveats with contusion injuries for studies of axon regeneration (discussed later).

A key factor for studies of axon regeneration is to be able to definitively identify regenerating axons and insure that they have in fact regenerated rather than being spared. The problem of spared axons has plagued SCI regeneration research, causing misinterpretations and claims of success that have later proved to be false. Criteria for identifying regenerated axons have been defined (Steward et al., 2003), and injury models differ in the extent to which axon regeneration can be definitively established.

Accordingly, this review summarizes the advantages and caveats of different rodent SCI models specifically for studies of axon regeneration. We summarize the pathways and regeneration assays that can be studied using particular models, functional assessments, caveats and disadvantages. This organization is complementary to the organization in a previous review (Tuszynski and Steward, 2012) where the perspective was regeneration of particular pathways and which injury models were most useful. It is important to note that the focus here is the injury models; examples of regenerative growth are discussed to illustrate the models, not to be a comprehensive review.

1.3. Spinal cord injury models for studies of axon regeneration

1.3.1. Complete injuries: surgical transections

Given that the potential for spared axons is a caveat for studies of regeneration, it follows that a desirable injury model is one in which one can be sure that all axons of a particular pathway are interrupted. This is one reason that complete transections are used.

The main advantage of complete surgical transections is that the lesions are relatively easy to create, do not require special equipment, and if properly done, one can be sure that all ascending and descending pathways are interrupted so there is no issue of spared axons. Complete transections are done via laminectomy at the level of choice using a surgical blade, scissors or a combination of the two. Complete transections can be done at high thoracic levels and lower, but not at higher levels because the overall impairment with a complete cervical injury would be unacceptable from an animal welfare standpoint. In recent studies involving rats, animal welfare was acceptable with complete transections at T3 (Lu et al., 2012; Sharp et al., 2014), but this is probably near the upper acceptable limit for complete transections.

Although it seems simple in principle to cut the spinal cord, in fact it is not easy to reliably make complete transections. If one simply does a laminectomy and draws a blade across the spinal cord, ventrally-located axons are usually spared. Because pathways that are important for hindlimb locomotion (reticulospinal and vestibulospinal tracts) are located in the ventral column, there can be considerable recovery of hindlimb motor function following lesions that were meant to be complete. It is important to take extra steps to ensure that ventral axons are transected, for example by cutting and then aspirating the contents of the lesion (see for example Lu et al., 2012). Even with additional steps, it is important to verify lesion completeness histologically on a case-by-case basis.

Complete surgical transections, as well as partial surgical transections, are not considered to be *clinically-relevant* models of human spinal cord injuries. Spinal cord injuries usually occur as a result of vertebral crush or displacement, which crushes the spinal cord or as a result of powerful concussive forces. The spinal cord retains its basic form after the crush, and the area of the crush undergoes degeneration leaving a scar/cystic cavity. In contrast, surgical transections are clean cuts with no concussive force. After the transection, the cut ends of the spinal cord draw apart, creating a separation that varies from animal to animal. At early post-lesion intervals, it is likely that the distance between the cut ends changes with movements of the animal. Later, development of a dense connective tissue scar likely limits movement of the spinal cord, and there may tethering of the spinal cord to the vertebral column.

It can be safely said that there will be no regeneration of CNS axons across the gap created by a complete surgical transection unless some bridge is provided such as a peripheral nerve graft, cell or tissue graft or scaffold. Hence, complete transection injuries offer advantages as a test bed for implants to enable axon regeneration. Axons seen within