Technical comments on rodent spinal cord injuries models

Spinal cord injuries (SCI) in rodents have been created by laceration, contusion, compression, or intramedullary injection of toxic agents. The choice of an appropriate SCI model should be made for each study based on the experimental design, with care taken to avoid unintended complications such as hemorrhage. Technical comments will be made in this communication describing the 1) importance of vertebral stabilization, 2) injury preparation, and 3) landmarks to improve the precision and reproducibility of the SCI. SCI models are often used to assess the spectrum of pathological events following spinal cord contusion, laceration, and compression injuries. Animal models are essential in understanding underlying injury mechanisms and determining the safety and efficacy of putative therapies prior to clinical trials. Most SCI models were initially described and characterized in the rat (Wraithall et al., 1985; Feihlings et al., 1989; Bresnahan et al., 1991; Stokes, 1992; Taoka and Okajima, 1998; Scheff et al., 2003; Zhang et al., 2008). These models offer unique opportunities to examine biological mechanisms following SCI, use of mouse models have increased exponentially (Burda et al., 2013; Hansen et al., 2013; Sabeltrom et al., 2013; Satkunendarajah and Feihlings, 2013; Soderblom et al., 2013; Takeuchi et al., 2013; Wanner et al., 2013; Zhang et al., 2013a; Zukor et al., 2013; Wu et al., 2014; Yamamoto et al., 2014; Ziu et al., 2014). Common rodent SCI methods are briefly reviewed in this report, and pertinent technical comments are made for those dedicated to SCI research. Nerve root and conus injuries will not be discussed.

SCI models

Contusion SCI is the most clinically relevant SCI. Key parameters used to determine the severity of SCI are different in various models such as the weight of the object and drop height (NYU (Gruner, 1992), others (Wraithall et al., 1985)), injury force (IH (Wraithall et al., 1985; Scheff et al., 2003)), calibrated spring tension (Gale et al., 1985), or magnitude of spinal cord displacement (ESCID (Stokes, 1992) or LISA (Zhang et al., 2008)). The NYU device has been extensively utilized on rats but does not produce reproducible lesions in mice. The IH device is simple to use, however, it creates a lesion with an impact velocity of only 0.1 m/s, which does not reflect that seen clinically. Gears in the IH device move in discrete steps, and is accompanied by a risk of injuries caused by mild forces. The signal-to-noise ratio is low using the IH device in mice. The ESCID and LISA spinal cord displacement models produce a satisfactory contusion SCI but require vertebral stabilization at the level of injury as well as precise displacement calibration. The modified aneurysm clip model is unacceptable because of variable clip blade locations applied to the spinal cord and the rate of clip release is not calibrated or standardized. There are advantages and disadvantages of each method, however, it is essential to stabilize the vertebra at the level of injury and create a precise, measureable, reproducible injury at a predetermined site. Over the past several years, our laboratory has developed the LISA system (Zhang et al., 2008) that discusses several of the shortcomings of currently available models. Specifically, the LISA answers the problems of vertebral stabilization, precise determination of displacement depth, and control and measurement of several parameters that address the impact reliability, specifically, the velocity, time, and duration of impact. Laceration SCI may be created by using microscissors, a razor blade, a needle tip, or a wire knife under manual control (Frisen et al., 1993; Hermanns et al., 2001; Imman et al., 2002; Seitz et al., 2002). Creating a precise laceration is difficult using these methods because of imprecise depth and shape of the laceration, bleeding, and unintended contusions. Variability of laceration injuries is often recognized only by postmortem microscopic assessment of the lesion site. Alternatively, our laboratory has developed a reliable, reproducible laceration spinal cord model in rats and mice by combining vertebral stabilization and the Vibranknife (LISA) which can generate laceration SCI in the cervical or thoracic spinal cords (Zhang et al., 2004; Oifer et al., 2005; Iannotti et al., 2006; Zhang et al., 2008; Hill et al., 2009; Yu et al., 2011). The oscillating blade of the Vibranknife cuts the spinal cord without tissue deformation. The depth of the laceration lesion is precisely controlled with an accuracy of ±0.05 mm using a micro-driver. There is minimal bleeding from the spinal cord using this method (Zhang et al., 2013b).

Compression SCI is produced by a decrease in the spinal canal diameter (Ziu et al., 2014), transplanting a synthetic aromatic polymer (Karadimas et al., 2013), inserting a solid spacer into the epidural space (Dimar et al., 1999), epidural spinal cord compression using modified aneurysm clips (Nashmi and Fehlings, 2001; Weaver et al., 2001; Fehlings, 2009), or creation of a static pressure from specific weights placed on the epidural space (Diamar et al., 1999). Common rodent SCI methods are briefly reviewed in this report, and pertinent technical comments are made for those dedicated to SCI research. Nerve root and conus injuries will not be discussed.

Comments

Technical contributions of producing SCI in rodents are designed to create a reproducible, well-characterized, and predictable model to analyze short- and long-term events following SCI (Kouyoudjian et al., 2009). Improvement in the quality of the SCI will reduce errors in the evaluation of SCI and avoid false conclusions following therapeutic studies.

(1) Secure target vertebral stabilization. The spinal column is flexible throughout its length which causes problems in creating a reliable SCI. Thus, spinal column stabilization is critical in producing predictable and reproducible experimental SCI (Kuhn and Wraithall, 1998; Zhang et al., 2004; Zhang et al., 2008; Hill et al., 2009; Zhang et al., 2013b). Two clamps fix the spinous processes above and below the intervening segment. However, this method is ineffective in stabilizing the short fragile spinous processes of the cervical or thoracic vertebrae in rodents. A large number of mobile segments beneath the impact lead to variable injuries as a result of different forces striking the spinal cord. The cervical and upper thoracic (C1, T1) laceration SCI may be created through the interlaminar spaces without concomitant bone removal. The cervical and upper thoracic (C1, T1) laceration SCI may be created through the interlaminar spaces without concomitant bone removal. The cervical and upper thoracic (C1, T1) laceration SCI may be created through the interlaminar spaces without concomitant bone removal.
movement of the spinal cord would create significant errors. Anterior displacement of the vertebra and spinal cord using the NYU impactor is detected by the rebound trajectory of the falling object. Several spine fixation devices are commercially available, including the Narishige’s ST-7 (Narishige International, East Meadow, NY) and the Spinal Adapter 68091 (RWD Life Science, San Diego, CA). The drawback is that these devices are too large to integrate with the NYU impactor. Our laboratory has developed small vertebral stabilization devices designed to use in rats and mice to prevent motion during the creation of the SCI (Zhang et al., 2004; Hill et al., 2009; Zhang et al., 2013b). Reduction of vertebral motion significantly improves the precision/reproducibility of the experimental SCI in rodents.

2) Level of SCI. The segmental level of the experimental SCI depends on the purpose of the research study. Cervical SCI constitute >50% of the injuries seen clinically and, therefore, the cervical SCI injury model is the most clinically relevant. Thoracic injuries are predominantly a white matter injury and are appropriate for studies of tract regeneration. Lumbar SCI are rare and occur clinically at the T11–L1 and L1–L2. They are a combination of upper and lower motor injuries, thus, are not a pure SCI (Magnuson et al., 1999). The choice of the segmental level also depends on considerations such as 1) avoidance of blood vessels to reduce hemorrhage, 2) decreased experimental mortality, and 3) increased post-SCI activity to lessen the burdens of post-injury care. Upper cervical spine surgery causes extensive bleeding of the C0 lamina that must be controlled with bone wax (Ethicon, Inc., Somerville, NJ). Bleeding from the epidural venous plexus during T1 or T12 laminectomies is easily controlled using topical thrombin soaked in Gel foam (Pfizer, New York City, NY, USA). Severe SCI (not partial SCI, hemicon traction, or hemisection) in the upper cervical levels is devastating and of little value as an experimental model. Lower cervical and upper thoracic (C2–T1) SCIs are better. The injury level is identified counting down from the prominent C2 spinal process (Zhang et al., 2013b). Lower thoracic (T10–11) levels can be identified by the direction that the spinous processes point inferiorly but T10, projects superiorly. The T1 and T10 spinous processes can be palpated through the skin. The importance of defining the lower thoracic vertebrae is vital to avoid the common mistake of performing a T1 laminectomy. The large laminar arches often lead to a laminectomy of T12, and T13, however, these levels expose the lower thoracic spinal cord, conus medullaris, and upper filum terminale. If unexpected excellent functional recovery occurs following lower thoracic SCI, it is likely that the incorrect segment was injured. A high degree of care is necessary to identify the correct segmental level at which to perform a SCI in an animal model.

Conclusion
Experimental animals share similar neurological deficits following SCI to those seen clinically and may undergo similar treatment options. High quality SCI research depends on assiduous attention to injury type, technical details, and anatomical landmarks.

Zoe Zhang1, Yi Ping Zhang2, Lisa B. E. Shields2, Christopher B. Shields2
1 Department of Neurosurgery, University of Minnesota, Minneapolis, MN 55455, USA
2 Norton Neuroscience Institute, Norton Healthcare, Louisville, KY 40202, USA

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Corresponding author: Christopher B. Shields, M.D., Professor, Norton Neuroscience Institute, Norton Healthcare, Louisville, KY 40202, USA, cshields1@gmail.com

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References

Figure 1 Depiction of spine motion following experimental SCI comparing stabilization at different levels. (A) Fixation (+) of the spinous processes two levels from the target level that includes four mobile segments causing a large amount of movement during impact. (B) Fixation (+) of the spinous processes one level from the target involving movement at two joints results in decreased motion. (C) Stabilization of the facets of the target vertebra (+) bilaterally most effectively prevents motion of the target vertebra.


