EDITORIAL

The quintessential nerve injury model**Daniel Umansky, MD, and Rajiv Midha, MSc, MD**

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NERVE surgeons and scientists have been attempting to create the ideal nerve injury model for decades, with a surge occurring after World War II.^{1–4} Seminal works of Kline and colleagues attempting to emulate both war and civilian injuries have investigated several animal models, namely dogs, rhesus monkeys, baboons, and chimpanzees,^{3,4} with detailed comparative results of different regeneration patterns and variability between different animal species when investigating gross and histological characteristics, neuroma formation, and nerve conductivity. These findings supported very early astute observations by Ramon y Cajal.⁵ Liu and colleagues⁶ demonstrated structural failure of freshly obtained cadaver nerves when stretched beyond 10%–20%, and provided one of the first stress–strain curves and histopathological consequences of stress-induced failure, creating a basis for the appreciation of injury pathophysiology. This work was later revisited by Sunderland and Bradley in human spinal nerve roots.⁷ Subsequent work by Lundborg and Rydevik⁸ demonstrated intraneural circulation and blood-nerve barrier disruption at even an 8% stretch, stressing the importance of microarchitecture's contribution in nerve injury models. The above classic papers used slow, graded stretch, and indeed, Hightet and Sanders⁹ speculated that the degree of nerve damage is dependent on the total tension applied rather than on the rate at which the injury is occurring.

Although a significant understanding of both the molecular processes of peripheral nerve regeneration as well as injury mechanism and histopathological processes has occurred,^{10–14} a single gold standard, verified, and repeatable injury model is still lacking. Furthermore, models have repeatedly used rodents for their primary investigative purposes for multiple reasons,^{15,16} including relatively low costs, even though they entail several limitations, such as small size, relatively short regeneration distance, species-specific neurobiological regenerative profile, faster regenerating capacity, and bifascicular nerves in contrast to polyfascicular human nerves. Still, attempting to prove translation into larger nonhuman primates¹⁷ as well as clinical treatment heavily relies on these smaller animal models. Moreover, a well-developed and accepted rodent nerve injury model would harness the powerful tool of ge-

netic engineering to decipher molecular and mechanistic pathways that may underlie the development of new therapies.

Mahan and colleagues have gathered significant experience in developing a stretch injury model in rodents that provides us with a better understanding of the biomechanical, histological, and possible outcomes. Their excellent work^{18–20} uses a rapid-stretch technique in an attempt to more closely emulate the mechanism of clinical nerve stretch injuries, thus providing a potentially better rodent model for closed traction injuries in humans. In their biomechanical results,¹⁸ sciatic rat nerves subjected to both slow- and rapid-stretch injuries showed threshold- and directional loading-susceptible ruptures, while nerve branch anatomy seemed the most important factor contributing to tensile strength, thus having clinical importance. Moreover, injuries appeared as discontinuous processes that actually fall into different elastic, inelastic, and rupture biomechanical patterns, a finding that further supports seminal observations by Sunderland and Bradley.⁷ In their subsequent histological results paper,¹⁹ in which they evaluate previous biomechanical damage patterns, in-depth graded injury was studied, demonstrating undulation straightening of axons and graded endoneurial injury concurrent with stretch severity, whereas injury to both epineurium and perineurium was minimal and did not predict transition from one grade to another. This finding supported previous conclusions made by Sunderland that the epineurium provides little resistance to stretch injury even under rapid stretching conditions. In their transition to a mouse model,²⁰ inelastic and elastic injuries were correlated with both behavioral and histological assays, further demonstrating neuroma formation (simultaneous regrowth of neural fibers and excessive fibrous scar tissue proliferation) in inelastic injuries. Finally, in their current publication,²¹ Mahan and colleagues report their results of traction injuries to the sciatic nerve in mice, evaluating their model in comparison with crush and transection injuries (with and without repair), and perform motor and sensory behavioral studies in addition to histological analysis. Their data demonstrate neuroma formation in the stretch injury group in comparison to other injury models.

Because the current paper is a continuation of the au-

TABLE 1. Nerve injury model: species comparison

Animal Species	Supported Lit & Available Publications	Translational Factor*	Genetic & Mechanistic Studies	Critical Nerve Gap (cm)†	No. of Targeted Nerve Sites Investigated	Ethical Approval	Price
Mouse	Abundant	Low	Very high	~0.5–0.7	High	Easy	Low
Rat	Abundant	Low	High	~1.3–1.5	High	Easy	Low
Rabbit	Abundant	Low	Moderate	~3	Moderate	Easy	Moderate
Cat	Moderate	Intermediate	Low	~3–4	Low	Moderate	High
Dog	Moderate	Intermediate	Low	~3–4	Low	Moderate	High
Pig	Moderate	Intermediate	Low to moderate‡	~4	Low	Moderate	High
Sheep	Sparse	Intermediate	Low	~4	Low	Difficult	High
NHP	Sparse	High	Low	~4	Extremely low	Very difficult	Extremely high

Lit = literature; NHP = nonhuman primates.

* Clinical resemblance through verified studies and assessment methodology.

† Critical nerve gap is defined as a nerve gap over which no recovery will occur without some form of nerve grafting or bridging when repair investigations are conducted.

‡ “Humanized” pigs.

thors’ line of investigation, more emphasis should have been given to their previous results, to provide the reader with some continuity of the work attempted, especially given that the initial work was done in rat models and the later work was done in transgenic mice. Although the authors have briefly noted that they have not encountered significant differences between the models used in terms of elasticity or rupture threshold,²⁰ except inelastic maximum strain injury—believed to represent injury technique differences and not intermodel differences—a more detailed account for using the later models would have been appreciated. Furthermore, the authors could have underlined the key differences in the two models, and why they have chosen to extend the work to mice, including pros and cons of the two rodent models in comparison to other larger-animal models (Table 1). In our experience, obtaining behavioral results in mouse models, especially evaluation of sensation using the von Frey monofilament testing technique, is difficult and potentially unreliable, a notion that is further supported by published literature.^{22,23} This is especially so, because a combination of pain and deaf-ferentation can mask results, as the authors have rightfully noted in their discussion. That being said, the authors’ histological section (as in their previous publications) has the strongest and most convincing results presented, deserving more emphasis than their behavioral analysis. Finally, use of this method, especially in the different stretch injuries, might be technically difficult to reproduce and warrants further validation by other research laboratories.

As we enter an era of fast translation from the bench to the clinic, we believe that Mahan and colleagues’ collective work can provide a fundamental basis from which future investigations are carried forward for studies on nerve injury, regeneration, and therapeutic modalities. The work in mice is especially important, because it permits the study of genetically altered animals harboring a potential transgenic mutation to allow a better appreciation of mechanistic interactions between Schwann cell and other cellular and molecular participants in the nerve injury and regeneration response.²⁴

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Disclosures

The authors report no conflict of interest.

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Response

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We thank Drs. Umansky and Midha for their insightful and considered editorial. As they point out, nerve stretch injury has long been known to cause severe dysfunction and pathophysiology; however, the study and repair of peripheral nerve injury is a field that has lacked standardization and application of a clinically relevant injury model. Most of the current animal research investigates the cues of successful regeneration. We would also benefit from understanding the mechanisms that lead to failed regeneration, which is a common clinical outcome after traumatic injury.

Drs. Umansky and Midha's discussion of the benefits and drawbacks of different animal models highlights a key issue that all preclinical research faces: translating animal findings into clinical relevance. Experimental animals are not humans and experimental injuries often fail to mimic

clinical injuries. Drs. Umansky and Midha suggested that a more detailed comparison between rodent models—rats in the initial work and mice in the current work—would be beneficial; as they point out, the greater transgenic flexibility of the mouse model was a clear benefit. Although it would be both enlightening and worthwhile to understand variation between rodent species in nerve biomechanics, nerve histology, and neurological recovery, we considered a detailed study that extended beyond our published statistical comparison to be a less pressing concern for the transition to transgenic animals that would more closely reflect humans.

We also agree that behavioral work such as von Frey testing can be challenging in rodent species, because similar monofilament testing has been interpreted as a test for both hypesthesia¹ and allodynia.² Our attempts to overcome that challenge, including Hargreaves testing,³ proved inconclusive. These results indicate that the contributions from both neuropathic pain and loss of afferent fibers are complex phenomena that require careful experimental design and data assessment, as ceaselessly expressed in the dynamic spectrum of pain and its perception that our clinical patients describe.

We wholeheartedly concur that further validation and advancement to larger animals is necessary and will provide new insights into the biomechanics and cellular response of polyfascicular nerves. Our rapid-stretch injury model is flexible and can be applied in both rodent and larger-animal models, much as controlled cortical impact, contusion weight drop, and fluid percussion have all become relatively standardized models for injury to the brain and spinal cord. To take advantage of the various benefits listed in the editorial's Table 1, we and our partners are currently using our stretch-injury model in both rats and larger animals for experiments in imaging, electrophysiology, and behavioral assessment to more fully answer biomechanical and physiological questions regarding nerve stretch injury, regeneration, and neuroma formation. We intend to continue our work in mice to take advantage of tools such as genetic sequencing and transgenic modification to probe cellular and molecular contributions to failed regeneration, with the goal of using this knowledge to develop therapeutic solutions for improving nerve injury outcomes.

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