Bipolar Electrocautery of Rodent Peripheral Nerve
A Model of Sunderland Type 3 Injury

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Abstract

Objective: Nerve injuries are a significant source of surgical morbidity. The prognosis after bipolar electrocautery of nerve tissue is uncertain, with management decisions based upon limited clinical and experimental data. To better define such injuries, we compared bipolar electrocautery-induced nerve injury to crush nerve injury in a rodent sciatic nerve model. **Subjects and Methods:** Twenty-two rats received sciatic crush (a reproducible Sunderland II) or bipolar electrocautery injury and were evaluated for functional, histomorphometric, and immunohistochemical recovery at 21 or 42 days. Axonal regeneration and endplate reinnervation were evaluated in double transgenic Thy1-CFP/S100-GFP mice. **Results:** Compared to crush injury, bipolar electrocautery injury caused greater disruption of myelin and neurofilament architecture at the injury site and decreased nerve fiber counts and percentage neural tissue distal to the injury ($p<0.05$). Complete functional recovery was seen after crush but not bipolar electrocautery injury. Serial live imaging demonstrated axonal regeneration at week 1 after crush injury and at week 3 after bipolar electrocautery. Qualitative assessment of motor endplate reinnervation at 42 days demonstrated complete neuromuscular endplate reinnervation in the crush group and only limited reinnervation in the bipolar electrocautery group. **Conclusion:** Bipolar electrocautery injury in a rodent model resulted in a Sunderland third degree injury, characterized by gradual, incomplete recovery without intervention.

Introduction

Bipolar electrocautery has proven valuable in decreasing the risk of iatrogenic damage to peripheral nerve associated with unipolar cautery. Nonetheless, the surgeon will occasionally encounter a patient in whom misidentified neural tissue was inadvertently cauterized. Previous research suggests that bipolar electrocautery induces perineural destruction similar to a Sunderland type 4 injury (Hnatuk LA, 1998), whereas a crush results in a more limited injury characterized by axonotmesis and Wallerian degeneration (Sunderland type 2 injury) (Bridge et al., 1994) **(Figure 1)**. Based on these observations, it was hypothesized that animals with a crush injury would demonstrate more robust nerve regeneration, less disruption of structural elements on immunohistochemical staining, and improved functional outcome relative to those animals with bipolar cautery nerve injury.

To compare the effect of crush and bipolar cautery on peripheral nerve we used the rat and mouse sciatic nerve model. The rat model allows for nerve histology and walking track analysis to monitor for functional recovery. The double transgenic Thy1-CFP/S100-GFP mouse allows for serial live imaging of the axon and Schwann cells (SC) over time following nerve injury, and allows for neuromuscular endplate staining to identify functional connectivity.

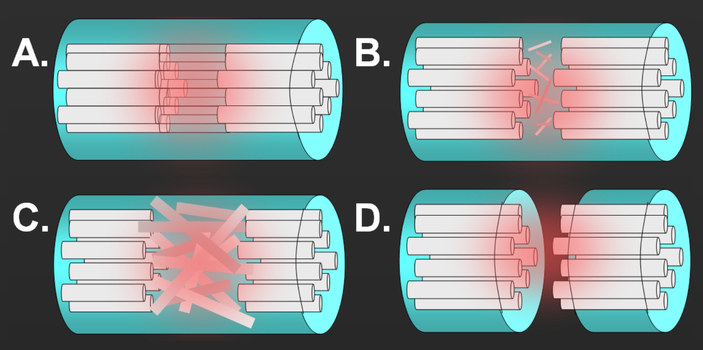

Figure 1. Sunderland classification of peripheral nerve injury. A: Second-degree. Axonotmesis. Disruption of axon continuity with intact endoneurial tubes. Spontaneous axonal regrowth and full recovery are expected. B: Third-degree. Perineurial continuity with axonal destruction and some internal disruption. Variable course, with slow incomplete recovery. C: Fourth degree. Total disorganization of internal structures, epineurium intact. Requires excision of damage and surgical repair. D: Fifth-degree. Neurotmesis. Total discontinuity of all neural structures. Requires surgical repair; full recovery is not expected.

Experimental Design

Species	Endpoint	Injury	Number	Evaluation
Rat	3 weeks	Crush	8	Walking track analysis Histomorphometry Immunohistochemistry
		Cautery	8	
	6 weeks	Crush	3	Walking track analysis Immunohistochemistry
		Cautery	3	
Mouse	6 weeks	Crush	3	Serial live imaging Neuromuscular junction staining Whole-mount nerve imaging
		Cautery	3	

* Cautery, as used here, refers to bipolar electrocautery injury

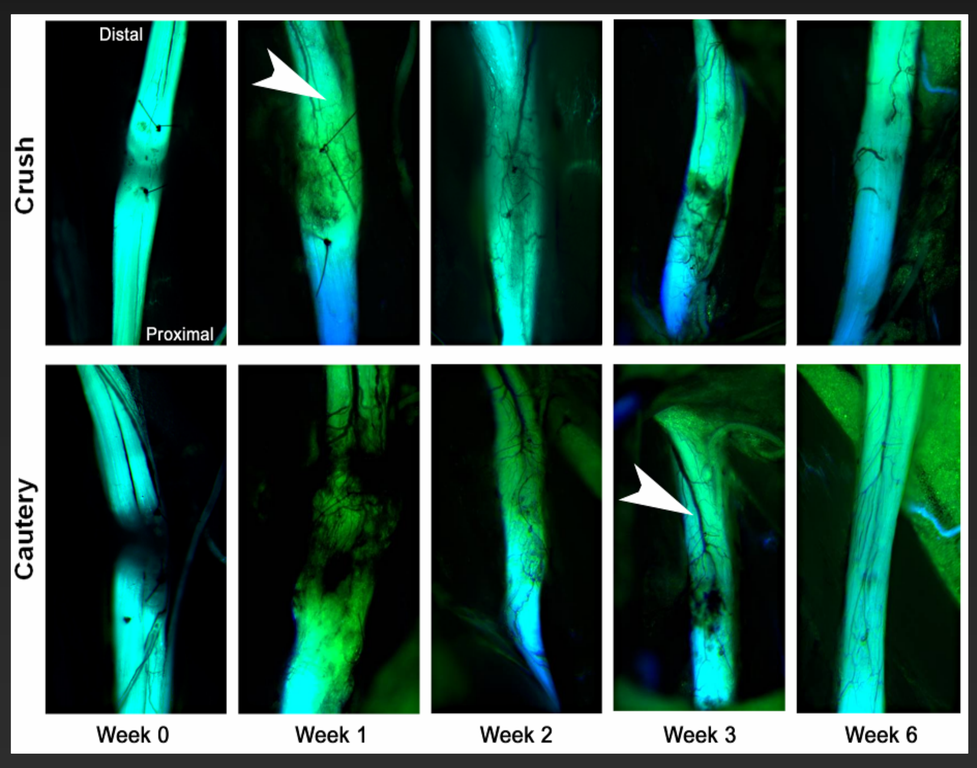

Figure 2. Serial live imaging. Blue: axons (Thy1-CFP). Green: Schwann cells (S100-GFP). Injury site is marked with proximal and distal sutures. Note that at week 1, axons have crossed the site of crush injury. In the cautery mouse, axons are not found distal to the injury until week 3.

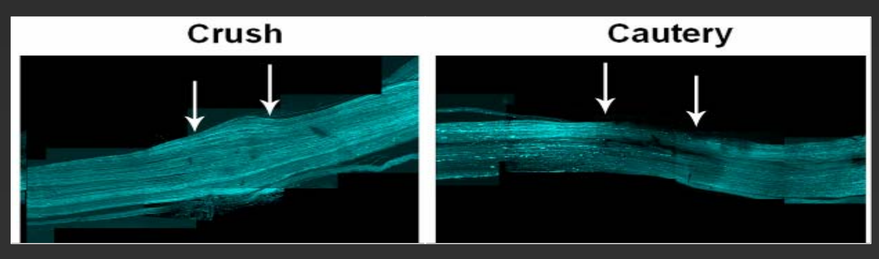

Figure 3. Whole-mount confocal images of transgenic mice demonstrate individual CFP-positive axons crossing the crush and cautery injury sites at 42 days. Arrows mark site of injury.

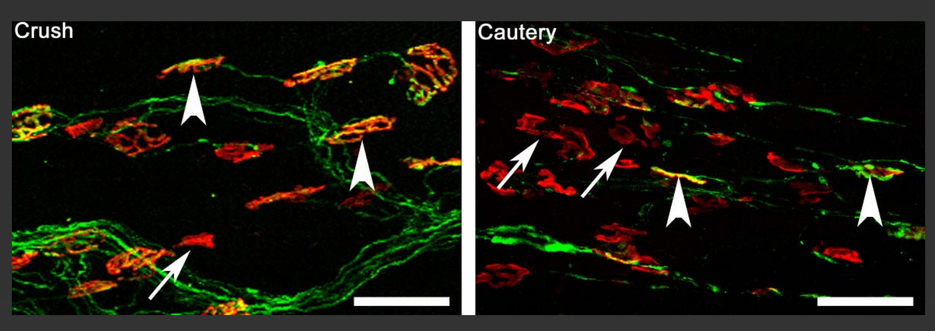

Figure 4. Neuromuscular junction (NMJ) staining in double transgenic mice. Arrowheads indicate reinnervated NMJs, distinguished by the yellow overlap between alpha-bungarotoxin staining (red) and native axonal CFP (green) ; arrows point to nonreinnervated NMJs. Scale bars are 50 µm. Specimens from mice receiving crush injury demonstrated reinnervation of nearly all NMJs; for illustrative purposes, a field containing a nonreinnervated junction is shown here.

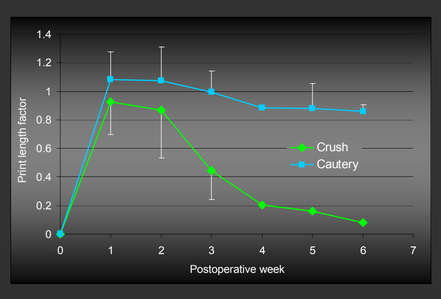

Figure 5. Walking track analysis. With crush injury, there is full functional recovery by six weeks. Significant functional impairment is evident in cautery-injured animals at six weeks, with gradual improvement continuing until the endpoint.

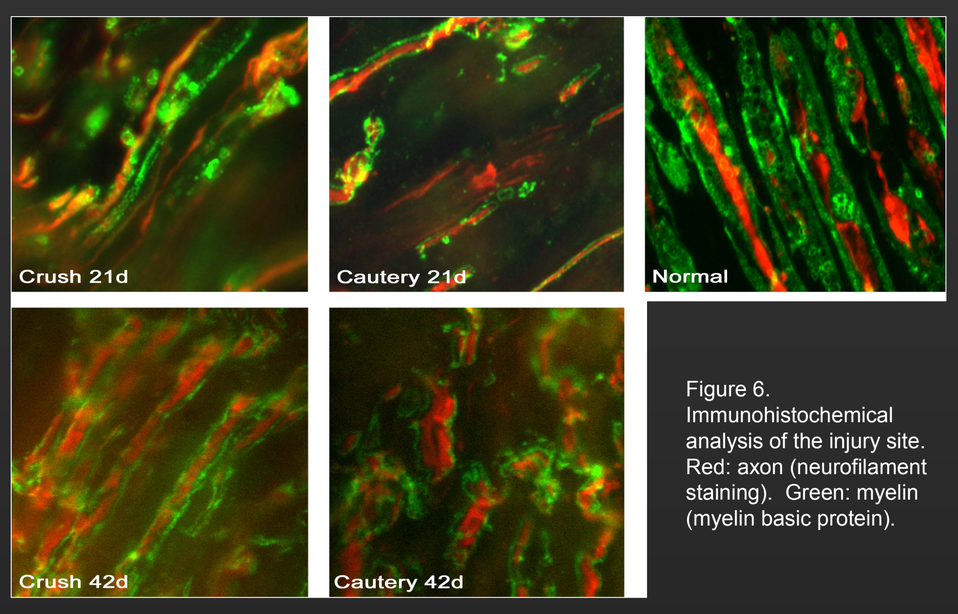

Figure 6. Immunohistochemical analysis of the injury site. Red: axon (neurofilament staining). Green: myelin (myelin basic protein).

Methods and Materials

Operative Procedures: Animals were anesthetized and the sciatic nerve exposed. Using a Bard System 5000 electrosurgical generator connected to a timer, nerves in the cautery group were injured by application of 30W of current (in coagulation mode) through microbipolar forceps for 1 second, approximately 5 mm proximal to the sciatic trifurcation in rats, and at a setting of 15W in mice. Nerves in the crush group were injured by firm application of the same microbipolar forceps tips without any current for 30 seconds. Injured sites were marked with 10-0 nylon epineurial sutures.

Serial live imaging: Double transgenic Thy1-CFP/S100-GFP mice were randomized to sciatic nerve crush or bipolar electrocautery. The incision was opened weekly and their injury site exposed and imaged with a fluorescence-enabled operative microscope. SC activity **(S100-GFP, green)** and axonal regeneration **(Thy1-CFP, blue)** were visualized for 6 weeks. At the endpoint, nerves were harvested for immunohistochemistry and muscle for confocal microscopy.

Walking tracks: The hind feet of the rat were coated with X-ray film developer, and the rat was allowed to walk down a undeveloped X-ray film track, producing hind footprints. Loss of sciatic nerve function results in unopposed dorsiflexion with corresponding elongation of the print length. The print length factor (PLF) is a normalized index of this impairment and drops over time as function is restored. PLF = (EPL – NPL)/NPL, where EPL and NPL are the print lengths of experimentally injured feet and unaffected feet, respectively.

Immunohistochemistry: At 3 and 6 weeks post-injury, the injury site in rats, was harvested along with 5-6 mm of surrounding nerve. Nerve segments were longitudinally sectioned and labeled with myelin binding protein (Chemicon - AB980) and neurofilament (Sternberger Monoclonals - SMI312) antibodies, then stained with the fluorophores Alexa488 (green) for MBP and Cy3 (red) for neurofilaments.

Results

Bipolar electrocautery injury of the sciatic nerve in the rat and mouse resulted in a Sunderland type 3 injury characterized by slow, incomplete recovery.

Mouse Model: Weekly serial live imaging of mouse nerves after crush injury reveals normal SC migration (green) and axonal regeneration (blue) starting at 1 week, complete by week 6. With bipolar cautery injury, robust SC migration is seen followed by late, partial axonal regeneration **(Figure 2/3).** Motor endplate staining of the extensor digitorum longus (EDL) muscle with confocal microscopy shows near complete reinnervation of motor endplates in the crush group. Minimal functional connections are seen in the bipolar cautery group; however, some endplates are reinnervated **(Figure 4)**.

Rat Model: Walking track analysis revealed complete functional recovery in rats after 6 weeks post-crush. In contrast, gradual, incomplete recovery was observed after bipolar electrocautery by the 6 week endpoint **(Figure 5)**. Immunohistochemical staining of rat nerves at 3 and 6 weeks demonstrated a greater disruption of myelin and neurofilament architecture at the bipolar electrocautery versus crush injury sites **(Figure 6)**. Histomorphometric parameters at the 3 week endpoint differed only slightly at the injury site between groups, but distally, nerve morphometry demonstrates significantly decreased total nerve fiber count, nerve density, and percent neural tissue, all $p<0.05$. Perineurial destruction was observed in nerve segments injured by bipolar electrocautery, but was not observed in segments injured by crush **(Figure 7/8)**.

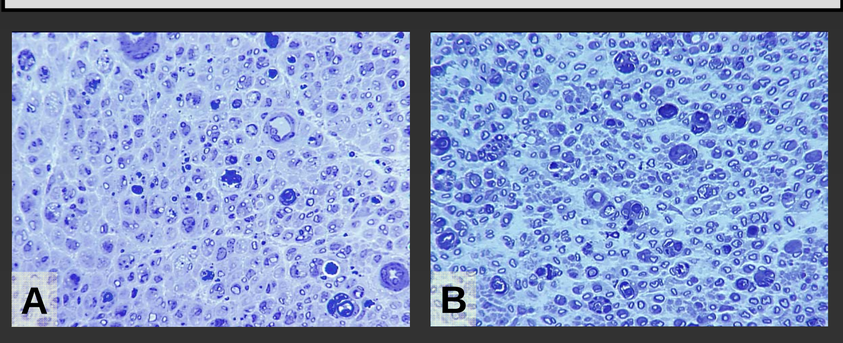

Figure 7. Qualitative histomorphometric findings at 3 weeks.
A: Distal to crush injury. B: Distal to cautery injury, with comparatively less regeneration and more damage-related debris evident. At 3 weeks post-injury, the sciatic injury site and a 1-cm segment of tibial nerve distal to the sciatic trifurcation were harvested and embedded in epoxy. 1-µm cross sections were stained with toluidine blue and evaluated with light microscopy. A digital image-analysis system linked to morphometric software was used to measure section attributes.

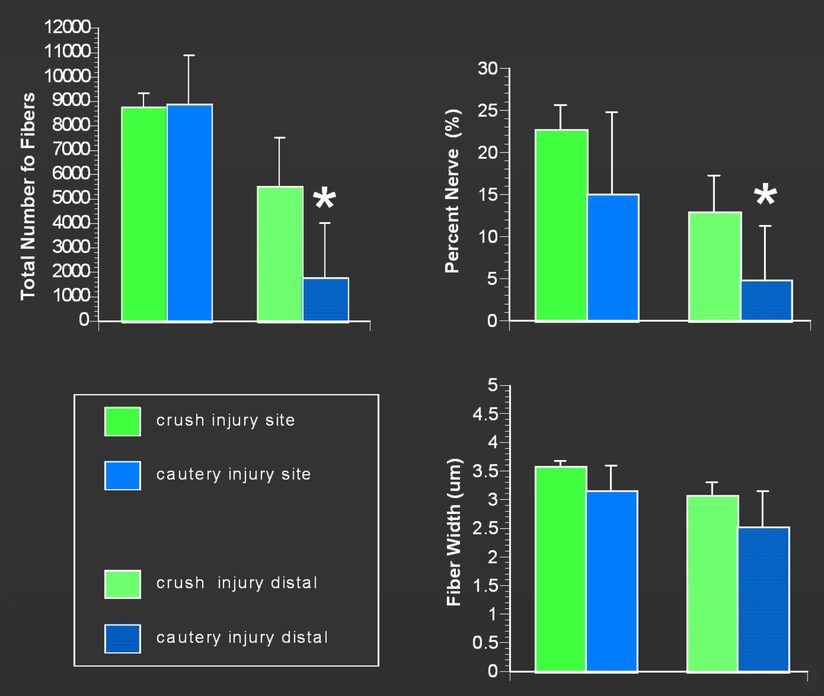

Figure 8. Quantitative histomorphometric findings at 3 weeks. A: Distal to crush injury. B: Distal to cautery injury, with comparatively less regeneration and more damage-related debris evident. C: Histomorphometric parameters. While there were no significant differences in parameters at the injury site itself, distal to the injury, the total number of fibers and percent nerve were significantly different ($p<0.05$, marked with asterisk).

Conclusions

The nerve crush model reliably produces a Sunderland type 2 (axonotmetic) nerve injury with slow, complete recovery. The present study demonstrates in the mouse and rat model that bipolar electrocautery results in a more profound impairment of sciatic nerve function than a standard crush model, with mild perineurial damage and spontaneous histomorphometric recovery at the injury site at 3 weeks, but significant residual functional impairment at 6 weeks. These functional and histological characteristics indicate that bipolar electrocautery induces a Sunderland third degree peripheral nerve injury in the rodent model; characterized by slow, variable, incomplete recovery.

We now have the first animal model of a third degree nerve injury which will advance peripheral nerve research. Type 3 injuries have the widest variability in recovery and thus, further studies are necessary to elucidate the effect on human nerve, ultimately with the goal of defining treatment paradigms for this uncommon but disabling injury.

References

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