Title: Functional and Histological Outcomes of Rapid-stretch Nerve Injury in the Mouse

Introduction:

Peripheral nerve injury accounts for 1-3% of all trauma cases, however, recovery remains limited despite progress in surgical intervention\textsuperscript{1,2}. In severe cases of traumatic nerve injury, persistent pain may even prevent complete engagement in therapy – compromising not only quality of life, but also restricting functional recovery\textsuperscript{3}. Unfortunately, current animal models employed to study peripheral nerve injury utilize transection, compression, and crush injuries\textsuperscript{4}. These standards fail to translate clinically, where a majority of traumatic peripheral nerve injuries are the product of a rapid-stretch mechanism\textsuperscript{5}. We sought to create a clinically relevant, rapid-stretch injury model in order to reproduce comparable pathophysiological damage in the laboratory setting, and thus establish an appropriate foundation to improve understanding and treatment of severe nerve injuries. We hypothesize that a rapid-stretch injury model will reveal structural damage more consistent with human injury pathology, and that this damage will reflect functional recovery outcomes.

Methods:

The sciatic nerve of C57BL/6 and B6.Cg-Tg(Thy1-YFP)HJrs/J male mice (N=38) was rapid-stretched using four biomechanically determined grades of injury: sham, elastic (less than 15\% persistent length deformation), plastic (permanent length change) and rupture. Behavioral tests included rotarod, walking analysis, tapered beam, Von Frey, and Hargreaves, at selected intervals for a duration of 6 weeks post-surgery. Histological analysis consisted of trichrome, osmium, and immunohistochemistry stains. Wet muscle weight of the gastrocnemius and tibialis anterior muscles was also obtained. All procedures were performed in accordance with protocols approved by the University of Utah IACUC.

Results:

Functional performance after injury correlated with rapid-stretch injury severity. For tapered beam (Fig. 1), the more severe injury grades (Plastic and Rupture) demonstrated a significant increase in footfalls immediately following surgery, and limited, yet progressive recovery ($p<.05$, Two-way ANOVA, Scheff’s post-hoc). Von Frey results (Fig. 2) also exhibit a drastic decrease in responsiveness post-surgery ($p<.01$, Two-way ANOVA, Scheff’s post-hoc), and a hypersensitive recovery for plastic injury. Histological analysis reveals a loss in traditional fiber undulations and increasing structural damage (microtears) with progressive injury severity (Fig. 3). Several nerves demonstrated the formation of neuroma-in-continuity: a pathophysiological outcome in human patients, yet a novel development within the laboratory framework. Gastrocnemius wet muscle weight of the injured limb revealed significant difference between all injury grades, excluding sham vs. elastic ($p<.01$, One-way ANOVA, Tukey post-hoc).

Conclusions:

Rapid-stretch injury has yielded consistent and reliable results across both non-survival and survival animal testing paradigms. Functional and histological analysis support the disparate
injury grades previously established by biomechanical analysis, and demonstrates recovery limitations of severe injury. The novel pathophysiological development of neuroma-in-continuity prompts a more clinically relevant animal model, and establishes the appropriate framework for imminent therapeutic strategies. Future studies include the addition of the clinically relevant EMG test and retrograde labeling to better discern conduction disparities and confirm the fidelity of reinnervation in the rapid-stretch model. Finally, the use of the Thy1-YFP mouse will permit in-vivo 2-photon imaging of the sciatic nerve at serial end-points, and provide further insights into the progressive regeneration of nerve architecture.

References:


Supplementary Materials

**Fig. 1.** Tapered Beam footfall analysis in injured hindlimb (L) (N=38). Comparison between baseline (-1) and up to day 2 in plastic and rupture injuries demonstrates a significant increase in footfalls ($p<.05$, Two-way ANOVA, Scheff’s post-hoc). Rupture and Plastic injuries are significantly different from each other throughout the entire testing paradigm, excluding baseline (Day -1) ($p<.01$, Two-way ANOVA, Scheff’s post-hoc).
**Fig 2.** Average # of responses to a 2.0g Von Frey filament in biomechanically determined injury grades (N=38). Comparison between sham and elastic groups is not significant, yet these groups demonstrate significance in comparison to both plastic and rupture injury grades ($p<.01$, ANOVA, Scheff’s post-hoc). There is a drastic decrease in responsiveness from D0 to D-1 ($p<.01$, ANOVA, Scheff’s post-hoc) for plastic and rupture. These injury grades are also significantly different from one another from D9 to testing completion ($p<.05$, ANOVA, Bonferroni’s post-hoc).
Fig 3. Longitudinal sections, sliced at 15μm, of (A) Sham (B) Elastic (C) Plastic and (D) Rupture injury grades subject to Lillie’s trichrome stain and imaged at 40x.