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Analysis of Regenerative Peripheral Nerve Interface Procedure with Neuroma Retention versus Neuroma Excision in a Distal Tibial Injury Rat Model

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Introduction

Peripheral nerve injuries, particularly those involving the distal limbs, often lead to the formation of painful and debilitating neuromas. These neuromas develop when severed nerve endings fail to regenerate properly, resulting in disorganized nerve growth that can cause chronic pain and functional impairment. The Regenerative Peripheral Nerve Interface (RPNI) is an innovative technique designed to prevent neuroma formation and promote nerve regeneration by inserting the severed nerve end into a muscle graft. While RPNI has shown promise in improving nerve regeneration, there is ongoing debate about whether neuromas should be retained or excised during the procedure. This study aims to assess the outcomes of RPNI with neuroma retention versus excision using a distal tibial injury rat model, with a focus on functional recovery, histology, and pain assessment.

Methods

Sixty male Sprague-Dawley rats were assigned to four groups: sham surgery, DTI with untreated neuromas, DTI followed by RPNI with retained neuromas, and DTI followed by RPNI with neuroma excision. Functional recovery, pain perception, and histological outcomes were evaluated using a series of behavioral tests (von Frey, hotplate, cold plate, CatWalk gait analysis, open field, and dynamic weight-bearing), electrophysiology, and histological examination. The study lasted 12-14 weeks, with initial DTI surgery performed at baseline and subsequent RPNI procedures conducted after 3 months. Additionally, 20 female Sprague-Dawley rats were used in the study and divided into two groups: sham-operated and DTI-operated. Figure 1 provides a schematic overview of the study design.

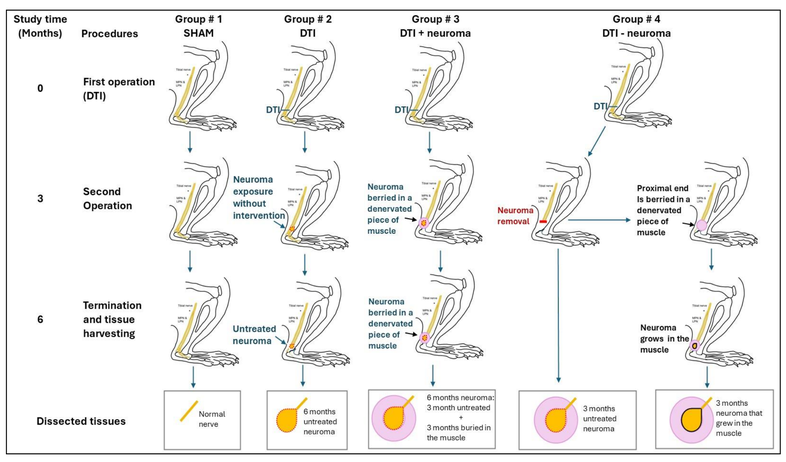

Figure 1. Study groups, procedures, and tissue collection.

Tests and Outcomes

Measure Outcome:	Outcome Measure	DTI vs SHAM	Neuroma Excision vs Retention	Summary
Pain-Related Behavior	Tactile Allodynia (vF assay)	Prolonged tactile allodynia in the DTI group	Minor effect **favoring excision**	1. DTI resulted in clear tactile allodynia with a moderate effect on weight-bearing. 2. The RPNI procedure had a minor effect in vF and DWB, **favoring neuroma excision.**
	Hot Plate (50-55ºC)	No effect	No effect	
	Cold Plate (0-4ºC)	No effect	No effect	
	Dynamic weight bearing	Moderate effect	Minor effect **favoring excision**	
Functional Assays	CatWalk: Print area	Clear and prolonged effect of DTI	Moderate effect **favoring neuroma retention**	1. DTI surgery resulted in expected gait changes between the operated and healthy paws. 2. RPNI had a minor to moderate effect, **favoring neuroma retention.** 3. There was no effect on the locomotor activity.
	CatWalk: Ratio of maximum contact area (Intact vs. operated leg)	Clear improvement in healthy leg vs. operated leg in DTI animals	Minor **favoring neuroma retention**	
	Locomotor Activity (Open Field Test: Walking distance)	No effect	No effect	
In Vivo Electro-physiology	Spontaneous firing from sciatic notch	DTI increased spontaneous firing frequency	Moderate effect **favoring neuroma retention**	1. DTI resulted in a variable yet statistically significant increase in spontaneous firing frequency. 2. RPNI procedure has a variable yet statistically significant effect **favoring neuroma retention.**
Histology	General Pathology and Gap43 Staining	Neuroma was clearly observed; differences noted between 3- and 6-month neuromas.	Muscle growth observed in both; neuroma excision group showed more order and less inflammation.	1. A clear neuroma was found post-DTI, with maturation of neuroma over time. 2. Histopathology **favored neuroma excision,** yet retaining the neuroma in muscle had some positive effects.

Results

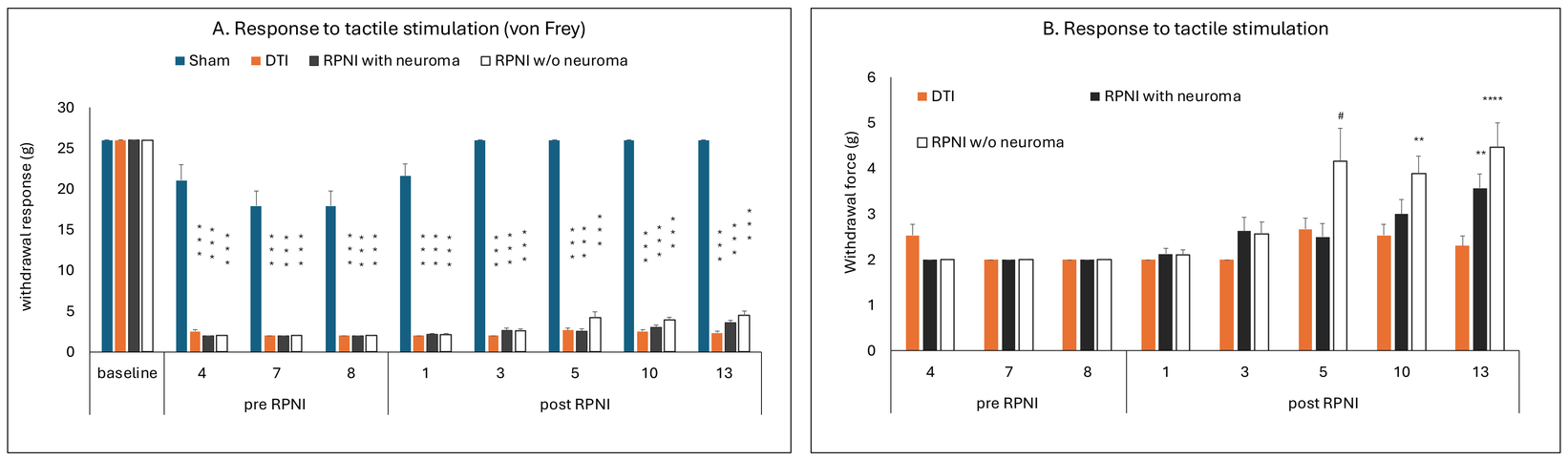

Figure 2. Response to tactile stimulation using the von Frey assay. (A) Following DTI, all animals showed reduced withdrawal force compared to baseline, indicating increased tactile sensitivity. Animals treated with RPNI showed a slight increase in withdrawal force at 10- and 13-weeks post-treatment. RPNI combined with neuroma removal (empty bars) appeared to show a slight advantage in recovery compared to RPNI treatment alone. (B) Animals treated with RPNI and neuroma removal exhibited a progressive increase in withdrawal force post-treatment. A significant difference was observed between RPNI with and without neuroma removal at later time points. ** p<0.01 vs. DTI Group;***p<0.001 vs. DTI Group; #p<0.05 vs. RPNI with neuroma Group.

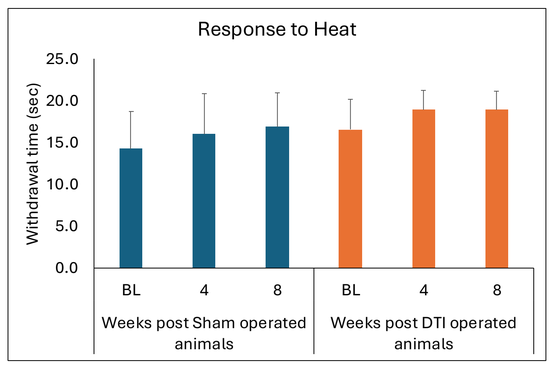

Figure 3. Response to Heat. Animals were exposed to heat (50-52ºC) using a hotplate apparatus. No significant differences were observed between baseline and post-operation responses. Additionally, there were no differences between sham-operated and DTI-operated animals.

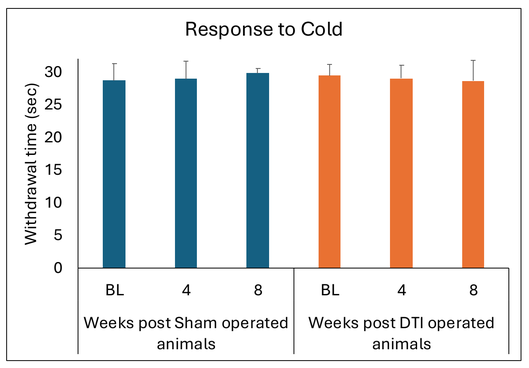

Figure 4. Response to Cold. Animals were exposed to cold (0-4°C) using a cold plate apparatus. No significant differences were observed between baseline and post-operative responses. Additionally, there were no differences between sham-operated and DTI-operated animals.

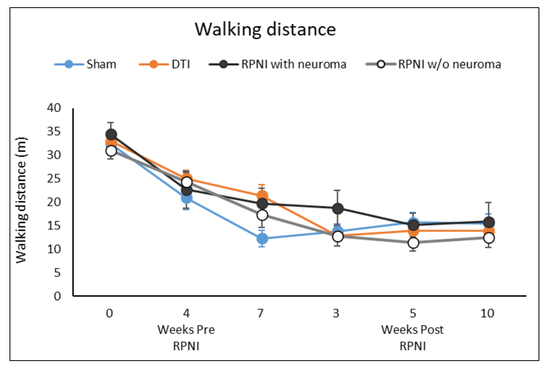

Figure 5. Open field assay. The open field test showed a comparable reduction in walking distance across all groups, with no statistically significant differences observed between them.

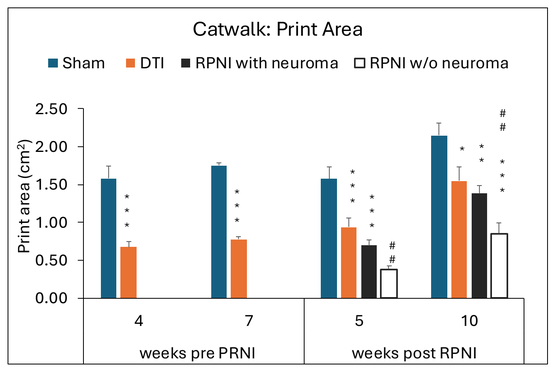

Figure 6. Hind paw print area. The hind paw print area of the operated leg (orange) was reduced following DTI. RPNI surgery involving neuroma removal resulted in a gradual recovery, as indicated by differences between groups (empty columns vs. black columns). *p < 0.05 vs. Sham animals; **p < 0.1 vs. Sham animals; **p < 0.01 vs. Sham animals; ##p < 0.01 vs. RPNI with neuroma.

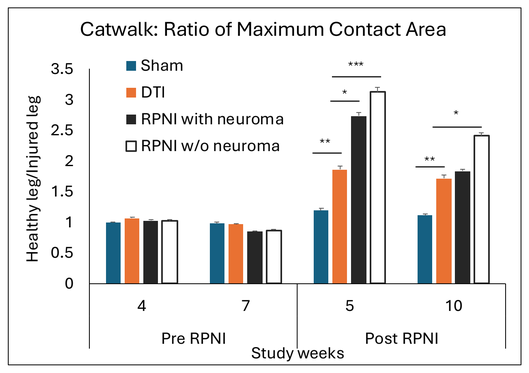

Figure 7. Hind paw ratio of maximum contact area. The ratio was calculated by dividing the maximum contact area of the healthy leg by that of the injured leg. Before RPNI surgery, no difference was observed between the two legs. At 5 weeks post-RPNI or after a second surgery in the DTI animals (orange columns), the maximum contact area of the injured leg was significantly reduced, resulting in the healthy leg having 2 to 3 times greater contact area. The RPNI without neuroma group exhibited the highest ratio of maximum contact area. ** p < 0.01 vs. Sham group; *** p < 0.001 vs. Sham group; # p < 0.05 vs. DTI group; ### p < 0.001 vs. DTI group.

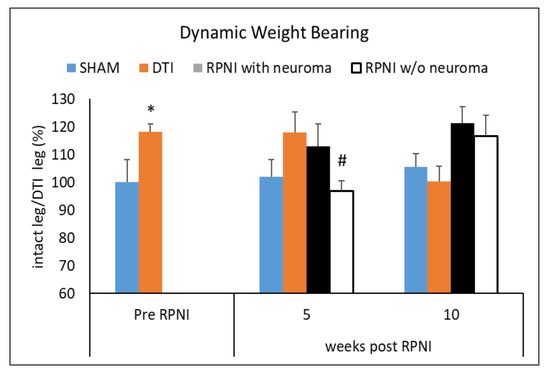

Figure 8. Dynamic weight bearing. The data is presented as a ratio (%) between the weight carried on the hind injured leg vs. on the intact hind leg. No statistical difference was observed between Sham-operated animals (blue bars) and DTI-operated animals (orange bars). Animals that underwent RPNI without the removal of the neuroma (black bars) showed similar ratios to the DTI group. Animals treated with RPNI including neuroma removal (empty bars) showed a beneficial effect 5 weeks post-operation compared to the the DTI-operated animals. *p<0.05 vs. DTI Group; #p<0.05 vs. RPNI with neuroma group.

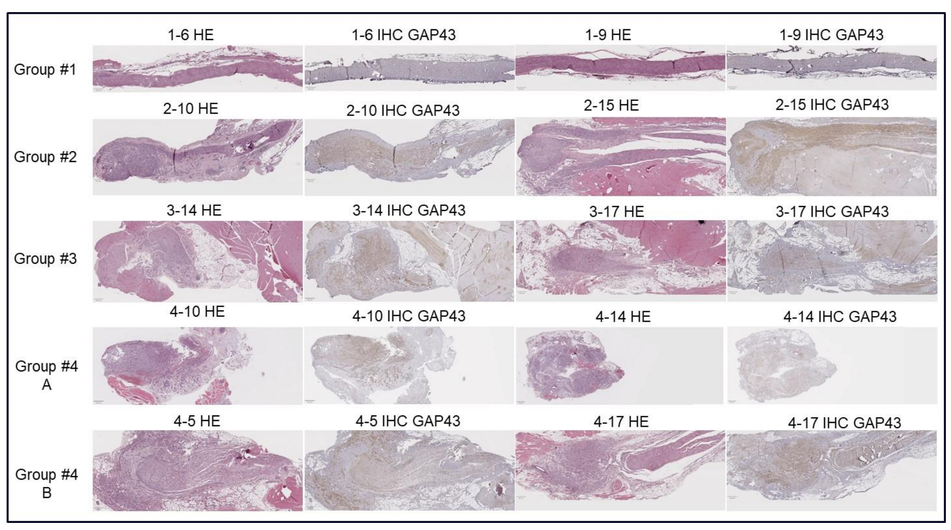

Figure 10. Histology images of a normal nerve and a neuroma.
Row 1 (Group #1): The naïve sciatic nerve structure shows normal architecture with H&E staining and low GAP-43 levels, as expected in untreated nerves. **Row 2 (Group #2):** Six months post-neuroma, severe axonal loss, and Schwann cell hyperplasia were observed, along with strong GAP-43 staining in the neuroma region and spindle cell fascicles. **Row 3 (Group #3):** A neuroma left untreated for 3 months and then buried in muscle for another 3 months showed moderate axonal loss, Schwann cell hyperplasia, and nerve fiber degeneration. Strong GAP-43 staining was present in the neuroma region and spindle cell fascicles, indicating nerve sprouting. **Row 4A (Group #4):** A 3-month-old neuroma exhibited severe axonal loss and Schwann cell hyperplasia. Nerve fibers showed Wallerian degeneration and were replaced by spindle cells, obliterating the perineurium. Strong GAP-43 staining was noted in the neuroma region, with moderate staining in the nerve. **Row 4B (Group #4):** A 3-month neuroma developed in the muscle after the untreated neuroma was removed, and the proximal end buried in the muscle. This resulted in a "quieter" neuroma with clear spindle cell fascicles growing into skeletal muscle. Strong GAP-43 staining was found in the neuroma region and spindle cell fascicles, with moderate to strong staining in the nerve.