INTRODUCTION:
Many individuals suffer from entrapment neuropathies, such as Carpal Tunnel Syndrome, that cause significant morbidity. While surgical decompression can be successful for treating many of the symptoms, the outcomes are quite variable and reversal of motor weakness is limited necessitating the need to understand these injuries. Previous studies of CNC injury showed that during the initial injury phase, there is a dramatic Schwann cell turnover in the absence of any evidence of axonal pathology. In the current study, we demonstrate that there is a decrease in myelination along with a decrease in internodal length with a corresponding loss in nerve conduction velocity over time in a murine model of CNC injury. In order to assess the possibility that the changes seen subsequent to compression are caused by inflammatory pathways, we looked for inflammatory cytokines Tumor necrosis factor alpha (TNF-α) and Interleukin-1 beta (IL-1β) via Western blot in comparison to the transaction injury model. In addition, we induced chronic nerve compression in slow Wallerian degeneration mice (WLDs). These mice have a slowed response to acute nerve injury with a delay in axonal degeneration and clearance of myelin debris. Our hypothesis is that if Wallerian degeneration plays a defined role in chronic entrapment, then these mice would be protected from the effects of compression.

METHODS:
Animal model creation: All animal protocols were approved by the Institutional Animal Care and Use Committee of the University of California, Irvine, Irvine, CA. Six-week, male C57BL/6 mice and were anesthetized and the sciatic nerves were exposed dorsally through a gluteal-splitting approach. The right sciatic nerve was subjected to chronic compression via inert tubing that was atraumatically placed around the nerve. The left sciatic nerve was mobilized and returned to its host bed to serve as a surgical control. For the transaction injury, the sciatic nerve was exposed through the same gluteal splitting approach. The right sciatic nerve was transected proximal to the bifurcation was performed and the proximal stump of the nerve was buried under the gluteus maximus musculature. The left sciatic nerve served as a surgical control. Weekly nerve conduction velocity (NCV) recordings were performed in vivo. Western Blot analyses of sciatic nerves for inflammatory mediators were performed to assess for inflammatory markers with comparison to the transaction injury model.

Morphometric Analysis: The sciatic nerves were harvested from experimental and control sides at 2 and 6 weeks after surgery. Nerve specimens were fixed, embedded in Spurr Resin, and cut in 1-μm sections. A montage of a normal nerve section (N=4), and a nerve section at the CNC injury site (N=4) were created and the g-ratios (ratio of axon to total fiber diameter to assess myelination) of all axons were calculated with a minimum of 1000 counted axons. For internodal length measurements, nerves underwent osmification and were dehydrated in ascending glycerin solutions and then teased into single fibers. Over 25 fibers were measured from C57BL/6 mice to assess myelination at two week and six weeks post CNC injury.

Statistical Analysis: Data are expressed as mean ± SEM and were evaluated by one way ANOVA followed by post hoc tests depending on experimental design. Differences among groups were considered significant if p<0.05.

RESULTS:
The average NCV in C57BL/6 mice and C57BL/6j/Wlds is 53.2m/s and 56m/s respectively. The average NCV decreased two weeks after injury to 42.6m/s and 35.8m/s in C57BL/6 mice and C57BL/6j/Wlds respectively, which declined to 27.2m/s and 31.4m/s by six weeks post-injury. The difference between the affected sciatic nerves of the two strains mice was also not statistically significant (Figure 1). There was an absence of detectable TNF alpha protein in the harvested nerve samples. Average normal g-ratio was 0.62 in both C57BL/6 mice and C57BL/6j/Wlds. There was a significant increase in the in the both groups. Average g-ratio 2 weeks post-injury was 0.719 in C57BL/6 mice and 0.68 in C57BL/6j/Wlds and increased to 0.792 in C57BL/6 mice and 0.77 in C57BL/6j/Wlds at six weeks post-injury signifying a decrease in myelination (Figure 2). Analysis of single teased nerve fibers from sciatic nerves of wild type mice showed a significant decrease (P<0.0001) in the intermodal length over the six week time course (Figure 3). Average intermodal length of normal nerve fibers was 633.5μm (± 15.4) which decreased to 473.6 μm (± 14.8) at the two week time period and to 358.4 μm at 6 weeks (± 23.6).

DISCUSSION:
Compressive neuropathies were considered to be variants of acute injuries. They are characterized by decreased conduction velocity in myelinated fibers with minimal loss in amplitude, localized demyelination and subsequent remyelination within the injury. The average nerve conduction velocity (NCV) in C57BL/6 and C57BL/6j/Wld mice decreased in a similar pattern over six weeks post-CNC injury with a similar corresponding increase in g-ratio, signifying a decrease in myelination. There was an absence of TNF alpha western blot expression in C57BL/6 mice. Internodal lengths decreased over six weeks in the C57BL/6 mice signifying re-myelination. These data support that CNC injuries are peripheral neuropathies that are not primarily mediated by inflammatory cytokines and Wallerian degeneration early after the disease. Further investigation with this novel murine model should prove to be quite useful to better understand acquired neuropathies.

Figure 1A and B: NCV of wild type (C57BL/6) mice (A) and WLDs mice (B). There is a significant decrease in conduction velocity when compared to the control side in both species. At the end of the six week time period the compressed sciatic nerve had an average NCV of 28.6m/s in wild type mice and 31.1m/s in WLDs mice. (Bars indicate standard error)

Figure 2: Nerve cross sections of wild type (A, B, and C) and WLDs (D, E, and F) mice. Figure 2A and 2D are from control samples of wild type and WLDs mice respectively. Figure 2B and 2E are from samples of wild type and WLDs mice respectively after 2 weeks of compression injury. Figure 2C and 2F are from samples of wild type and WLDs mice respectively after 6 weeks of compression injury. In both wild type and WLDs mice there is a decrease in the level of myelination of axons as indicated by the arrows. (Scale bar: 10μm)

Figure 3: Teased Nerve fiber from a normal wild type sciatic nerve (A) and from a wild type sciatic nerve six weeks post compression injury (B). There is an increase in the frequency of nodes (arrows) in the compressed nerve segment. (Scale bar: 100μm)