

# Inhibition of SARM-1 Reduces Neuropathic Pain in a Spared Nerve Injury Rodent Model

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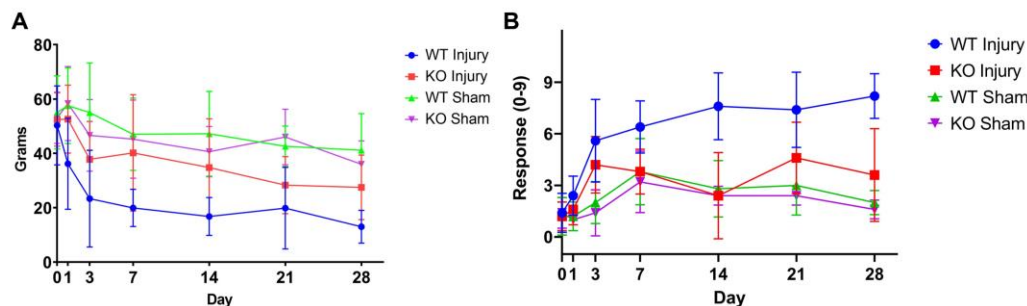
**Introduction:** The role of the SARM-1 protein has been shown to play an essential role in regulating axonal degeneration and its depletion halts this destructive process. Because Wallerian degeneration may contribute to the development of neuropathic pain, we hypothesize that loss of SARM-1 function will decrease neuropathic pain in a spared nerve injury model.

**Methods:** This study was approved by an IACUC and IRB committee. Thirty-two wild type (WT) or SARM-1 knockout (KO) rats underwent spared nerve injury (SNI) with peroneal and tibial nerve ligation and transection or sham surgery with no nerve injury (eight per group). Neuropathic pain was assessed using an electronic Von Frey aesthesiometer applied to the sural nerve distribution of injured and uninjured paws and the required force to stimulate withdrawal was recorded in grams. Nociception was evaluated at baseline and after surgery at predetermined timepoints (day-1, day-3, week-1, week-2, week-3, and week-4). A separate acetone test to detect cold allodynia was also completed. Nerve samples were qualitatively examined by light microscopy. Results were analyzed by one-way ANOVA followed by Tukey honestly significant difference tests for multiple comparisons.

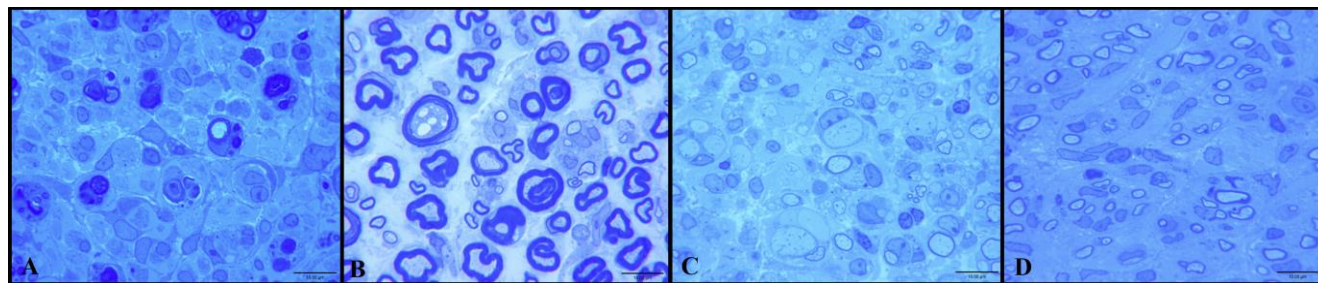
**Results:** Injured SARM-1 KO rats demonstrated a clear trend of more reduced sensitivity to nociceptive stimulation in a neuropathic pain model than the WT injury cohort, which reached significance at week-4 ( $13.0 \pm 6.0$  vs.  $27.5 \pm 11.5$ ,  $p=0.044$ ) (Figure 1A). Injured SARM-1 KO rats also showed less sensitivity to cold allodynia than WT Injury rats at week-2 and week-4 ( $p=0.002$ ,  $p=0.002$ ) (Figure 1B). WT Injury rats demonstrated greater sensitivity to pain than WT Sham at all timepoints (day-1, day-3, week-1, week-2, week-3, and week-4), validating the pain model ( $p=0.028$ ,  $0.002$ ,  $0.005$ ,  $<0.001$ ,  $0.002$ ,  $<0.001$ ) (Figure 1A). WT injury rats similarly showed greater reactivity to cold allodynia than WT sham at day-3, week-2, week-3, and week-4 ( $p=0.021$ ,  $0.003$ ,  $0.006$ ,  $<0.001$ ) (Figure 1B). However, KO Injury rats demonstrated no difference in pain or cold sensitivity compared to the sham cohorts at any timepoint. Light microscopy suggests reduced demyelination in KO rats compared to WT at site of nerve injury (Figure 2).

**Discussion:** We found that knockout of SARM1 lessens response to mechanical pain stimuli and cold allodynia after SNI, particularly at our final follow-up of 4 weeks. Qualitative results of light microscopy suggest attenuated distal axonal degeneration with limited macrophage infiltration in SARM1 KO rats. Our results support a mechanism of decreased exogenous macrophage response in SARM1 KO rats secondary to inhibited Wallerian degeneration ultimately resulting in reduced neuropathic pain.

**Significance:** Amid growing evidence that SARM1 plays an important role in axon degeneration, our findings suggest that targeting SARM1 may hold promise as a therapeutic approach for management of neuropathic pain.



**Figure 1A)** Nociception in SNI or sham treated paws of KO and WT rats measured in grams by electronic Von Frey. The WT Injury group showed a greater trend towards sensitivity of noxious stimuli at all timepoints than the SARM-1 KO Injury group and the sham cohorts. **Figure 1B)** Response to cold allodynia on either the SNI or sham treated paw of KO and WT rats measured by the acetone test. Severity of response was graded as no response (0), paw flick (1), repeated paw flick (2), repeated flick with lick (3). Measurements were made in triplicate and summed at each timepoint for total score of 0-9 with averages for each group displayed in this figure. Results demonstrate a trend of increased cold sensitivity in WT Injury rats not observed in the other cohorts.



**Figure 2)** Light microscopy at 1000x of peroneal nerves distal to nerve ligation within the injury site following SNI. **A)** WT injury peroneal nerve 2-weeks following SNI. **B)** KO injury peroneal nerve 2-weeks after SNI. **C)** WT injury peroneal nerve 4-weeks following SNI. **D)** KO injury peroneal nerve 4-weeks after SNI.