Peripheral and Central Nervous System Markers of Neuropathic Pain in a Chronic Rotator Cuff Injury Model

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INTRODUCTION: Pain management in patients with chronic rotator cuff injury (RCI) remains under-treated: recent studies have shown that up to 30% of rotator cuff tear patients are on long-term opioid therapy, even after surgical repair, including 23% who were opioid naïve pre-operatively. This suggests that the current treatment options are perhaps not targeting the correct—or the only—type of pain. Thus, in this study, we employed a chronic rotator cuff injury model to elucidate the contribution of neuropathic mechanisms to the composite pain milieu resulting from RCI.

METHODS: All of the following experiments employed the use of 10-12-week-old, C57/BL6 mice. To avoid sex as a confounding variable, we chose to first investigate RCI in male mice. To induce RCI, we performed a TT+DN injury (N = 6/group), defined as a tendon transection (TT) of the supraspinatus and infraspinatus tendon with a suprascapular denervation (DN). We sought to validate this model by (1) confirming functional deficit using a string-pulling assay (Figure 1A), where mice are trained to utilize their forelimbs to pull string downwards in a vertical motion and (2) assessing pain through forelimb weightbearing using the Blackbox R4 device (Blackbox Bio), as previously described [2]. In a separate experiment, mice underwent the identical TT+DN injury (N = 3/group). After 6 weeks, cervical spinal cord and bilateral cervical dorsal root ganglia (DRG) were harvested, sectioned, and stained for markers of neuronal injury: ATF3, Iba1, and c-FOS. In the last experiment, mice 10 weeks post-TT+DN injury (N = 7), were administered IP gabapentin (30mg/kg), a known neuropathic pain medication. Isolated forelimb grip strength was tested at baseline and post-gabapentin administration (Figure 3A). All data is reported as mean difference ± SEM. Significance was set as $p < 0.05$.

RESULTS SECTION: At 10 weeks, mice with TT+DN injury exhibited significant deficits in string pulling reaching motion, compared to naïve control, -56.93 ± 14.50, $p < 0.01$ (Figure 1B). TT+DN mice also experienced significant pain, as evidenced by decreased forelimb weighbearing ratio, -0.41 ± 0.11, $p < 0.01$, indicating placement of more weight on the uninjured, contralateral side (Figure 1C-D). Immunofluorescence histology showed signs of neuronal injury, with elevated markers of ATF3 and Iba1 in cervical DRGs of TT+DN mice, in addition to elevated markers of Iba1 and c-FOS in the cervical spinal cord, when compared to naïve controls. Lastly, gabapentin significantly improved isolated forelimb grip strength ratio (right/left), compared to baseline, 0.30 ± 0.06, $p < 0.001$ (Figure 3B).

DISCUSSION: In this study, we validated a chronic rotator cuff injury model in producing persistent functional deficit and inducing chronic pain. Additionally, we investigated the role of neuropathic pain showing evidence of neuronal injury in the cervical DRG and spine of injured mice. Lastly, we exhibit the analgesic role of gabapentin, a neuropathic pain medication, in an evoked pain grip strength assay.

SIGNIFICANCE/CLINICAL RELEVANCE: Here, we show evidence of clear neuronal injury detected in the peripheral and central nervous system, following rotator cuff injury. The alleviating effects of gabapentin suggest that neuropathic pain medications should be considered as first-line treatments for patients with chronic pain secondary to rotator cuff injury.

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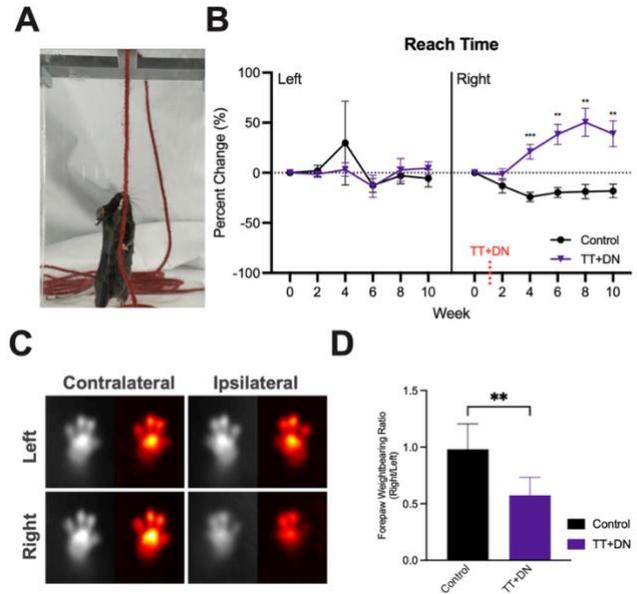
Figure 1. (A) Live image of string-pulling assay. (B) Percent change from baseline of left and right reach time. (C) Representative paw heatmap of bilateral paws in the injured (ipsilateral) and uninjured (contralateral) forelimbs, with increased red/orange indicating more weightbearing. (D) Forepaw weightbearing ratio at 10 weeks post-TT+DN.
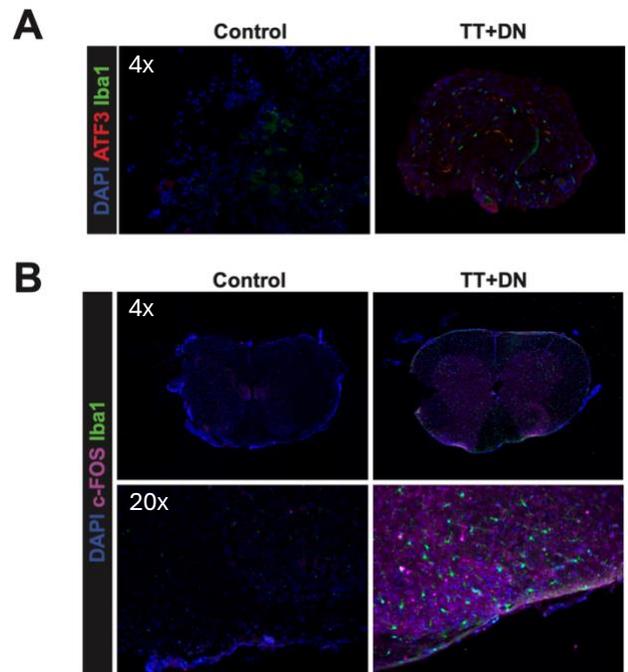

Figure 2. (A) Immunofluorescent images of cervical DRG sections from control and TT+DN mice showing elevated signs of neuronal injury (ATF3, Iba1) in the TT+DN cohort. (B) Immunofluorescent images of cervical spinal cord at 4x and 20x showing elevated signs of neuronal injury (c-FOS, Iba1) in the TT+DN cohort.
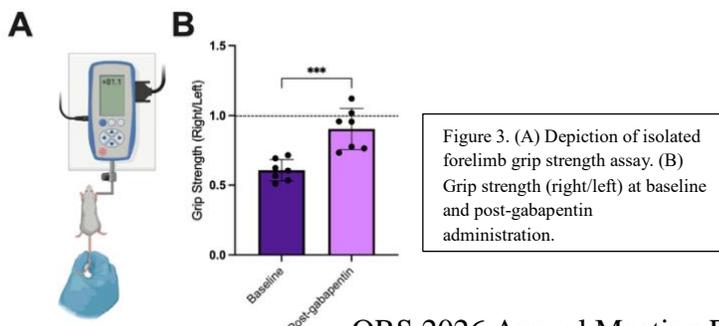

Figure 3. (A) Depiction of isolated forelimb grip strength assay. (B) Grip strength (right/left) at baseline and post-gabapentin administration.