Cortical Signatures of Chronic Pain after Rotator Cuff Injury in Mice

Sonali Feeley[1], Karnika Bhardwaj[2], Madison Jewell[2], Alex Youn[3], Xuhui Liu[3], Brian Feeley[3], Allan Basbaum[2], Jarret Weinrich[4]

[1]University of California, Los Angeles and University of California, San Francisco (UCSF) Summer Research Training Program, [2]UCSF Department of Anatomy, [3]UCSF Department of Orthopedic Surgery, and [4]UCSF Department of Anesthesia and Perioperative Care

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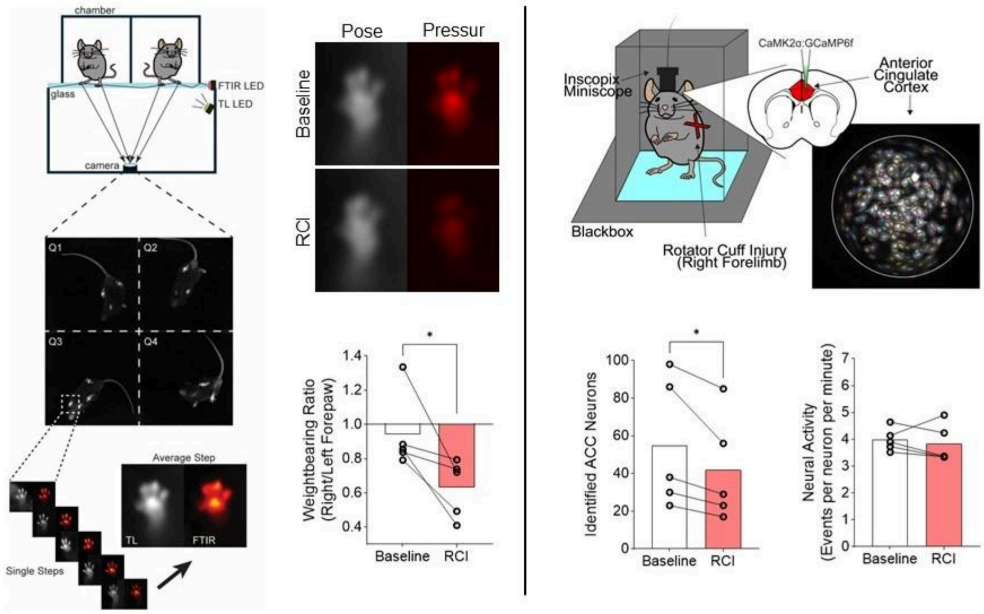

RCI produces quantifiable changes in pain behaviors and brain activity
<u>Left:</u> Schematic of procedure for Blackbox behavioral monitoring and data processing. RCI induces a decrease in weightbearing on the injured limb, as shown in average paw images as a decrease in the weightbearing ratio.
<u>Right:</u> Schematic of pair Blackbox and calcium imaging recordings with the Inscopix miniscope. After RCI, there is a decrease in the number of identified neurons but no change in average activity of neurons that continue to fire.

Introduction: Patients with rotator cuff injuries (RCI) often experience persistent chronic pain. RCI patients have limited treatment options, often involving repeated surgical interventions, and for which there is limited success in reducing pain. Traditional pharmacotherapies, such as oral anti-inflammatories (i.e., NSAIDs) or opioids, provide some pain relief, but many patients continue to experience pain that is not well controlled. Therefore, there is a significant unmet need for analgesic therapies that can effectively treat pain after RCI, and orthopedic injury in general. Importantly, <u>pain is experienced in the brain</u>, and we hypothesize that the lack of efficacious treatments for chronic orthopedic pain, such as that after RCI, is due to the lack of the understanding of the central neural drivers of chronic pain in these conditions. Here, our aim is to understand how RCI injury manifests as pain. We present preliminary data where we monitor activity of the anterior cingulate cortex (ACC), a region in the cerebral cortex that encodes for the affective-motivational (i.e., unpleasant) aspects of pain, before and after the induction of RCI, with concurrent deep behavioral phenotyping using the Blackbox system.

Methods: In adult male mice (N = 5 mice), to model massive RC tears, mice received unilateral SS and infraspinatus tendon transection with denervation (TT+DN) (Milan et al, 2025), and longitudinally assessed pain-related behaviors and gait patterns at baseline (prior to RCI) and 2 weeks post RCI. We used the BlackBox One system to perform high spatial and temporal recording of pain behaviors in freely moving mice, which captures both animal pose (body position) and paw weightbearing (paw pressure). The grayscale images produced are the transmitted light illumination of the hindpaw that captures animal pose and the heatmap images indicate paw pressure of frustrated internal reflectance (FTIR) with red indicating less pressure and yellow/white indicating greater pressure. We then performed automated extraction of hindpaw position and gait kinematics using Paw Analysis Workflow (PAW), and AI-enabled software developed by our lab (Layne et al, 2025). While monitoring behavior in the Blackbox, we simultaneously captured neural activity in the anterior cingulate cortex (ACC) via calcium imaging using an Inscopix miniscope (nVoke) (Weinrich et al, 2025). Under the control of the CamK2a promotor, GCaMP6f, a genetically encoded calcium indicator (GECI), was virally delivered into the ACC. A gradient index lens (GRIN lens) was then chronically implanted into the ACC. Mice are allowed to recover for 4 weeks before imaging sessions begin. During imaging sessions, mice were placed into the BlackBox and allowed to move freely. Data streams from the BlackBox and miniscope are synced via TTL pulse. Neural activity was extracted using Inscopix Data Processing Software (IDPS) MATLAB API, and custom written software. All procedures are performed in accordance with our approved IACUC protocol.

Results: After RCI, we observed clear changes in the behaviors of the mice. Specifically, there was a decrease in weightbearing on the injured forelimb, quantified as the forelimb weightbearing ratio (injured/uninjured forelimb; baseline: 0.94 ∓ 0.10, RCI: 0.63 ∓ 0.08, $p \leq 0.04$, paired student's t-test). In addition, we also observed shifts in the neural activity of the ACC after RCI. After RCI injury there were fewer active neurons than at baseline (baseline: 55 ∓ 15 neurons, RCI: 42 ∓ 13 neurons, $p \leq 0.04$, paired student's t-test). Interestingly, the average activity of identified neurons did not change (baseline: 4.0 ∓ 0.2 events/minute, RCI: 3.8 ∓ 0.3 events/minute, $p \leq 0.57$, paired student's t-test).

Discussion: Our results demonstrate that our novel approach to behavioral phenotyping has the sensitivity to detect changes in behavior after RCI. Detecting pain after forelimb injury in preclinical models has been notoriously difficult and laborious, however, using our AI-enabled deep behavioral phenotyping approach we are able to rapidly and reproducibly detect changes in forelimb function after injury while minimizing user bias. More importantly, we are able to couple our behavioral findings to activity changes in a brain region that encodes the unpleasantness of pain (i.e., the ACC). As the results presented above are preliminary, we are continuing to record behavioral and neural activity at later timepoints in an effort to further map the behavioral and neural correlates of the chronification of RCI pain. Additionally, we plan to assess how pharmacologically diverse analgesics alter ACC function and provide relief from RCI pain. Lastly, we plan to extend our studies to female mice to identify sex specific changes. Our current studies lay the foundation for identifying novel, efficacious treatments for RCI pain, which, hopefully, can translate to other models of orthopedic injury.

Significance/Clinical Relevance: Effective treatments for pain after rotator cuff injury are limited. Our study demonstrates that rotator cuff injury alters pain processing in the brain of mice, creating a preclinical neural biomarker against which novel pain relieving treatments for RCI can be screened.

References:

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