Assessment of Rodent Gait Relative to Pain as a Means of Evaluating Fracture Recovery

Emily McCarthy[1], Sarah Kirk[1], Moya Priddy[1], Matthew Ludington[2], Keith Irobosa[3], Paola Divieti Pajevic[3], Louis Gerstenfeld[2], Beth Bragdon[2], and Elise Morgan[1]
[1]Department of Biomedical Engineering, Boston University, Boston, MA 02115, USA.
[2]Department of Orthopaedic Surgery, Boston University Chobanian & Avedisian School of Medicine, Boston, MA 02118, USA.
[3]Department of Translational Dental Medicine, Boston University Henry M. Goldman School of Dental Medicine, Boston, MA 02118, USA.
eamcc@bu.edu

Disclosures: Emily McCarthy, Sarah Kirk, Moya Priddy, Matthew Ludington, Keith Irobosa, Paola Divieti Pajevic, Beth Bragdon, and Elise Morgan have nothing to disclose. Louis Gerstenfeld is a member of the ORS Collaborations Council.

INTRODUCTION: Rodent models are standard for studying fracture healing and its underlying molecular mechanisms. However, current preclinical assessments of bone healing largely depend on ex vivo imaging and mechanical analysis, which fail to capture the functional recovery and pain-related behaviors that are used clinically to assess healing. In rodent studies, the inability to directly measure pain presents a major barrier for predicting the full translational impact of a treatment [1]. Tools that quantify behavioral indicators of pain in rodents, such as altered gait or hunching, could overcome this barrier. The goal of this project was integrating quantitative gait analysis and posture detection through a machine learning pipeline, moving beyond traditional reflex-based methods (e.g., Von Frey) or ex vivo analysis [2]. This approach provides a shift from ex vivo imaging and mechanical assessment toward a more holistic, behavior-informed understanding of fracture healing in preclinical studies that better represents clinical outcomes.

METHODS: A structured longitudinal study involving 40 C57/Bl6J mice (8-12 weeks old, 20 female and 20 male) was performed. The animal study was approved by the institution's IACUC. The MouseWalker system included a GoPro Hero 12 high-speed camera, RGB LED light box, mirror, and a frustrated Total Internal Reflection (fTIR) walkway to precisely track mouse paw placement during locomotion. A second camera was added to track the lateral movement of mice, including rearing behaviors and posture. After baseline data was collected, the right hind leg received a closed femoral fracture fixed by intramedullary stabilization. Pain was assessed using Von Frey filaments testing for allodynia [3]. All outcome assessments were recorded at days 7, 14, 21, and 35 after surgery. At each time point, 8 mice were euthanized for concurrent radiological, histological, and molecular assessments of healing. During data collection, each mouse completed 4 uninterrupted walks across the walkway. After a walk was selected using standardized criteria (consistent forward locomotion, absence of rearing, and visibility of all limbs throughout the video), the ventral view video captured by the GoPro was converted to a series of grayscale images which were analysed in MATLAB after adding a gamma correction to ensure image compatability with the existing MouseWalker GUI [4]. Analysis produced quantitative gait parameters including stance offset, step cycle, and step distance, that were then compared across the progression of fracture healing. We further developed a novel image-based approach using a posture scoring system based on MacLellan et al [5] for postural analysis to separately provide quantitative measurements of pain-related behaviors using a ML model created in DeepLabCut [6]. The ML model takes as input lateral view video of the mice walking uninterrupted across the walkway and predicts the position of three key points along the neck and upper back region indicative of overall posture as an indicator of pain that can be correlated with gait measurements and allodynia. The model was trained on 190 frames and achieved a test RMSE of 3.09 pixels (1.51 mm) on unseen videos. Gait parameters and the hunched angle calculated between the three key points were averaged for one walk across the walkway for each mouse and compared across the fracture healing process using repeated measures ANOVAs (RM ANOVAs) followed by the Tukey test for post-hoc comparisons.

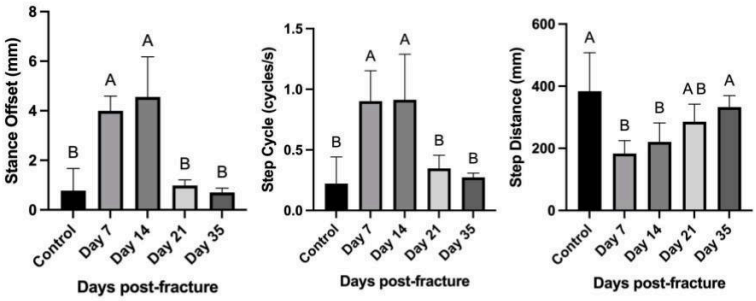

Figure 1: Gait parameters across fracture repair. Stance offset (Left), Step cycle (Mid), and Step distance (Right) return to baseline after initial significant increase following fracture.

RESULTS: All gait parameters showed significant differences between the control and days 7 and 14. By day 21 post-fracture, mice gait patterns return to baseline (Control) after showing initial and significant differences in gait parameters due to injury (Figure 1). Pose analysis revealed increased hunching (i.e. decreased angle) post-injury with the most intense hunching occurring 7 days post-injury and a significant increase in hunching through day 21 despite gait parameters having returned to baseline (Figure 2). This increased hunching corresponded to allodynia as determined from Von Frey testing, indicating that hunched posture in mice was an accurate indicator of pain.

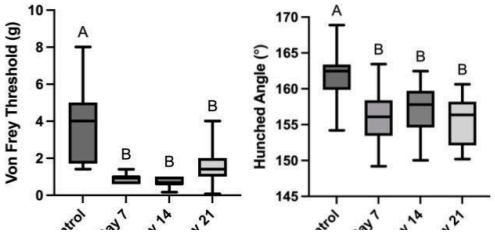

Figure 2: Comparison of pain measurements across fracture repair. (Left) Von Frey scores for the fractured limb and (Right) averaged hunched angle from modified MouseWalker both show significant changes in pain through day 21 following fracture.

DISCUSSION: By modeling gait parameters and patterns over time, we observed notable changes in movement that corresponded with the expected progression of recovery, supporting our design goal of enabling longitudinal assessments of fracture healing through a minimally invasive automated system. The lateral camera to assess pain through hunching provides a novel quantitative image-based surrogate for assessing pain in the context of functional regain of limb use. The focus on a shift towards comprehensive, quantitative assessment of murine behaviors in preclinical studies of bone fracture healing is adaptable to a variety of preclinical models including osteoarthritis, neurodegenerative diseases, or even post-operative recovery assessments where motor deficits and/or pain-related behaviors are critical data points.

SIGNIFICANCE: This study paves the way for a more holistic and behavior-informed understanding of the regain of limb function over the time course of fracture healing in preclinical studies, using approaches that more closely reflect clinical observations.

REFERENCES: 1. Bizzoca, et al, Injury, 54, 1, S46-S52, 2023. 2. Campana, et al, Methods in Molecular Biology, 1230, 229-231, 2015. 3. Notartomaso, et al, Bio Protoc, 8(2), e2671, 2018. 4. Mendes, et al, BMC Biology, 13, 50, 2015. 5. MacLellan, et al, Royal Society Open Science, 9, 11, 2022. 6. Mathis, et al, Nat Neurosci, 2018.

ACKNOWLEDGEMENTS: This work was partially supported by the Evans Center for Interdisciplinary Biomedical Research ARC on "Musculoskeletal Health across lifespan" at Boston University.