Novel, Rapid AI-Based Behavioral Phenotyping Identifies Effective Analgesics for Chronic Pain after Rotator Cuff Injury in Mice

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INTRODUCTION: Ineffective pain management in the setting of chronic rotator cuff injury (RCI) remains a critical issue [1]. Current treatment options; including NSAIDs, physical therapy, and surgical repair; fall short of providing patients with long-lasting analgesic relief, in turn contributing to poorer function and overall diminished quality of life. Therefore, there is an unmet need to identify more novel effective analgesic therapies to treat RCI pain. The goal of this study was to employ novel, AI-based tools to rapidly screen a pharmacologically diverse set of analgesics, some clinically relevant—morphine, an opioid, and gabapentin, an anticonvulsant—and others non-traditional—ketamine, a dissociative anesthetic, analgesic, and antidepressant; and 2,5-dimethoxy-4-iodoamphetamine (DOI), a psychedelic hallucinogen—in providing pain relief in a chronic murine RCI model.

METHODS: To record and measure longitudinal alterations in murine shoulder function, we used the Blackbox R4 device (Blackbox Bio), as previously described [2] (Figure 1A–C). At Baseline, 10, 3-month-old C57/BL6 mice were inserted individually into a dark, acrylic chamber and allowed to roam freely. During recordings, mice are illuminated by two near-infrared (NIR) lights: (1) a transillumination (TL) light to visualize overall body pose, and (2) frustrated total internal reflectance (FTIR) light to visualize paw contact pressure with the borosilicate glass floor (Figure 1A). Simultaneously, a high temporal and spatial resolution NIR camera records the mouse's behavior (Figure 1B). To induce a massive rotator cuff injury, all mice underwent a right shoulder TT+DN injury, defined as a tendon transection (TT) of the supraspinatus and infraspinatus tendon with a suprascapular denervation (DN). Recordings were then repeated at 2-, 4-, 6, and 8-weeks post-TT+DN injury. All mice were then randomized to each receive a total of five injections throughout the duration of the experiment: PBS (30mg/kg) (vehicle control), DOI (2mg/kg), ketamine (12mg/kg), morphine (10mg/kg), and gabapentin (30mg/kg). Injections were spaced out by a minimum of three days. All recordings were processed through Paw Analysis Workflow (PAW), a machine learning enabled software developed by our laboratory for analyzing paw weightbearing and kinematic parameters (Figure 1C) [2]. A linear mixed-effects models was applied to perform statistical analyses. Significance was considered when $p<0.05$. All data are presented in the form of mean ± SE. All statistical analyses were computed in GraphPad PRISM (Version 10.4).

RESULTS SECTION: To assess pain, and consequentially pain relief, we measured forepaw weightbearing ratio (ipsilateral/contralateral). At Baseline, the mice exhibited an average weightbearing ratio of 1.0 ± 0.10, denoting approximately equal forepaw weight distribution pre-TT+DN injury. After RCI injury (8 weeks post-TT+DN), there is a clear signature of ipsilateral limb pain resulting from the injury: specifically, there is significant decline in ipsilateral/contralateral weightbearing ratio, compared to baseline (1.0 ± 0.10 vs. 0.57 ± 0.06, $n = 10$/group, $p \leq 0.05$) (Figure 1D-E). In clear contrast, the RCI-induced decrease in weightbearing ratio was reversed by the administration of DOI ($0.57 \pm 0.06 \pm$ vs. 0.81 ± 0.08, $n = 10$/group, $p \leq 0.05$), ketamine ($0.57 \pm 0.06 \pm$ vs. 0.82 ± 0.09, $n = 10$/group, $p \leq 0.05$), morphine ($0.57 \pm 0.06 \pm$ vs. 0.81 ± 0.07, $n = 10$/group, $p \leq 0.005$), and gabapentin ($0.57 \pm 0.06 \pm$ vs. 0.77 ± 0.08, $n = 7$/group for gabapentin, $p \leq 0.05$ (Figure 1D-E), suggesting an analgesic effect encouraging mice to bear more weight on the ipsilateral, injured forelimb. Additionally, we observed a slight, yet significant, increase in the weightbearing ratio after the injection of saline ($0.57 \pm 0.06 \pm$ vs. 0.65 ± 0.07, $n = 7$/group for saline, $p \leq 0.05$).

DISCUSSION: In this study, we employed AI-based behavioral phenotyping to characterize a chronic murine RCI injury model, longitudinally track persistent pain, and ultimately display significant analgesic improvement with drugs, both commonly and uncommonly provided to treat RCI-associated pain. Traditionally, testing pain in mice is laborious, and this significantly impedes our ability to screen analgesics. However, now using our machine learning tools we can assess the pain state of an animal within minutes, without user intervention. In the future, we plan to use our methodology to screen effective analgesics for chronic RCI pain, thereby expanding the repertoire of drugs to identify most efficacious analgesic treatments, in addition to exploring potential sex differences in pain sensitivity and response to analgesics.

SIGNIFICANCE/CLINICAL RELEVANCE: The current array of treatment options for patients experiencing chronic RCI-associated pain remains insufficient. Our study suggests that a wider selection of analgesics can provide effective pain relief for these patients.

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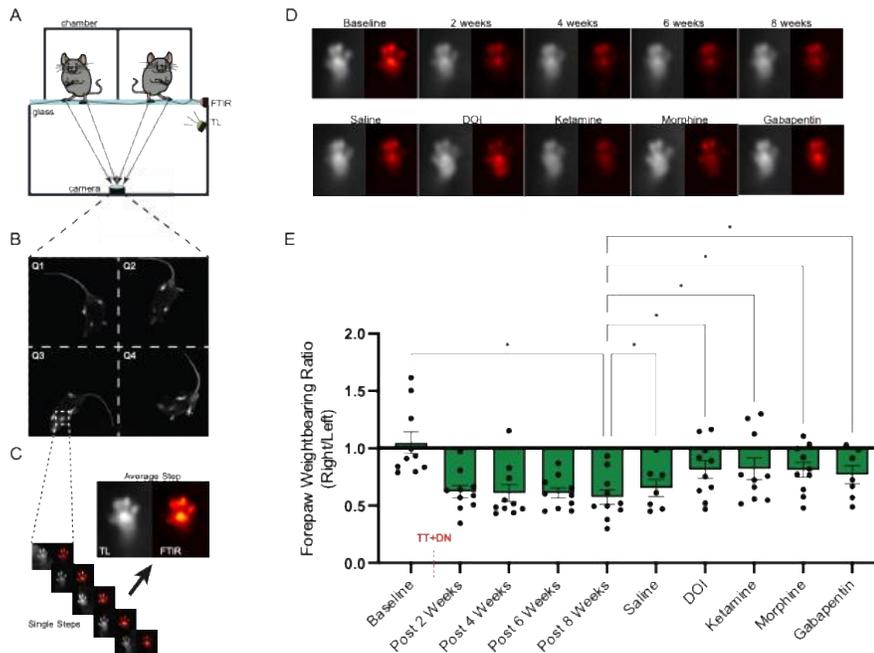
Figure 1. (A-C) Blackbox setup schematic: four-chamber arena, NIR camera, TL and FTIR LED strips. (D) Representative plantar heatmap of ipsilateral (right) mouse forepaw, with red/orange colors denoting higher applied pressure. (E) Quantification of forepaw weightbearing ratio (right/left) before and after analgesic delivery post-TT+DN.