

A fully-automated behavioral pipeline for reproducible pain assessment in mice

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Introduction

- 1. Understanding how individuals express their pain on a reflexive and affective, brain-driven level may lend crucial insights into delivering personalized treatments for pain disorders.
- 2. Furthermore, tools to measure pain are robust and established, yet many lack the resolution to dissect the behavioral underpinnings of paw withdrawal at the sub-second timescale.
- 3. Here, we introduce our latest iteration of Pain Assessment at Withdrawal Speeds (PAWS), a tool to evaluate naturalistic responses to evoked pain assays. With flexibility in mind, we demonstrate the capabilities of our **user-friendly interface for PAWS** analysis.



An interactive graphical user interface enables flexible PAWS analysis without programming experience.		
PAWS Dashboard	■ Optimize smoothing filters by assessing tracking	g trajectories in a CSV:
Y Run PAWS diagnostics Nun PAWS analysis ✓ Visualize pain scores ★→ Visualize PAWS kinematics	Upload a CSV and enter video parameters. Upload a tracked CSV (DeepLabCut or SLEAP) here: Browse Example_6J_female_lp_arm.csv Upload complete Enter the video frames per second (fps) here: 2000 Enter the measured distance (mm) between your reference points here: 40 If you aren't tracking reference points, enter a manual mm/px scale factor: 0.15	Select body parts, axes to track trajectories. <pre>choose a bodypart to graph: toe center heel Choose an axis to graph: x y</pre>
		Adjust filters to find an optimal filter and level of smoothing. Sovitation of the length:



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Conclusions

- 1. Key-point tracking using markerless tracking models (SLEAP, DeepLabCut) allows unbiased quantification of reflexive and affective responses to innocuous and painful stimuli.
- 2. With or without programming experience, PAWS diagnostics and smoothing optimization capabilities offer fine control over kinematic analysis.
- 3. Reflexive and affective features alone or in combination are sufficient to separate touch from pain-like responses.



PAWS analyzes videos from 2000-250 frames per second.

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