Advanced Dynamic Weight Bearing as an Observer-Independent Measure of Acute Pain
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INTRODUCTION

- Traditional methods to assess pain in mice are observer-dependent, criticized for their subjectivity, experimenter interference, and measure of stimulus-evoked pain only. 2
- Previous studies responses to mustard oil-induced pain showed no time-of-day dependencies. Although, these conclusions were based on results of observer-dependent measures.
- Alternatively, Advanced Dynamic Weight Bearing (ADWB) is observer-independent, unbiased, non-invasive, and an objective measure of non-evoked pain responses, the most clinically relevant type of pain.
- Here, we characterize the utility of ADWB in assessing responses to chemically induced acute pain in mice.
- To our knowledge, we are the first to use ADWB to assess behavioural responses to mustard oil-induced acute pain.

METHODS

Mice. Male inbred C57BL/6J mice 7–10 weeks old (24–30g) were used in all experimental procedures, all of which were approved by Queen’s University Animal Care Committee in accordance with the Canadian Council on Animal Care guidelines.

Mustard Oil Injections. The left hind paw was injected with 20 μL of 0.1% mustard oil or saline solution at Zeitgeber time (ZT)-2 (09:00) and ZT-14 (21:00) and were assessed immediately post-injection for a standard data acquisition period of 5 minutes.

Acute pain assessment. Responses to acute noxious chemical-evoked pain were assessed by Advanced Dynamic Weight Bearing (ADWB) while simultaneously, paw licking and biting time was recorded using a stopwatch.

Data analysis. ADWB software automatically detected and labeled position of all 4 paws and other body parts, which was manually and blindly verified. Standard low and high weight thresholds are 0.8g and 1.0g, respectively, with stable surface threshold minimum and maximum number of image values separately set to 2 and 3 (set 1); in that order, our adjusted parameter values were set to 0.3g, 0.5g, 2, and 2 (set 2). Experiment data was assessed frame-by-frame along timeline bar.

Statistical analysis. Data was analyzed with one-way ANOVA using Sigma Plot. Spearman rank order correlations were used to assess the relationship between observer-dependent and -independent data.

RESULTS

Figure 1. Parameters impact variability mean and validated data acquisition time, but not weight. ADWB data was analyzed first using set 1 and repeated using set 2. A) Differences in validated data acquisition time (s) were significant (p<0.001). B) Variability means were significantly different (p<0.001), but C) L/R Ratios did not differ between parameter sets (p<0.001).

Figure 2. ADWB detects changes in weight and surface area in mustard oil-treated mice. Mice that received mustard oil reduced the weight (g) (A) and surface area (cm²) (B) in their front and rear ipsilateral paws to their contralateral paws and other body parts (p≤0.001). Weight and surface area expressed as % of total front or rear paw weight and surface area, or body weight (other) in L/R groups. **p<0.01, ***p<0.001.

Figure 3. ADWB software-computed weight ratios. A) Ratio of ipsilateral (left) to contralateral (right) paw weights. B) Ratios of weight between front and rear paws. p≤0.001 (mean yellow line), median (black line). ***p<0.001.

Figure 4. Pain responses to mustard oil-treated mice. A) L/R footprint of ipsilateral paw (light brown). B) Mustard oil paw weight (B) was greater than saline and its contralateral paw (A).

CONCLUSIONS

- ADWB detection parameters impact length of validated time and result variability; future studies should consider this for data analysis.
- ADWB revealed a significant shift in body weight to favor the front paws and contralateral side, indicative of acute pain localized to the rear ipsilateral paw.
- Findings were consistent with our observer-dependent test showing increased licking and biting of rear ipsilateral paw.
- Data from ADWB measurements suggested that weight is a stronger measure of acute pain than surface area.
- ADWB assessed pain over time to reveal mustard oil-induced pain is most intense during the first 30 seconds post-injection.
- Mustard oil-induced pain exhibited no circadian rhythm.

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REFERENCES