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Advanced Dynamic Weight Bearing as an Observer-independent Measure of Hyperacute Hypersensitivity in Mice

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ABSTRACT

Background: Standard methods assessing pain in rodents are often observer dependent, potentially resulting in biased outcomes. Advanced dynamic weight bearing (ADWB) offers an observer-independent approach that can provide objective, reliable data in preclinical pain research.
Aims: The aim of this study was to characterize the use of ADWB in assessing murine responses to allyl isothiocyanate (AITC)-induced hyperacute hypersensitivity and identify best practices for use of the device.
Methods: Male C57BL/6J mice received intraplantar injections of saline or 0.1% AITC solution and were assessed using the ADWB system; simultaneous observer-dependent durations of paw licking and biting were measured. ADWB data were analyzed using the proprietary software from Bioseb and correlated to observer-dependent results, with parameters assessed to optimize data collected.
Results: ADWB detected pain-directed changes in weight and surface area distribution in AITC-treated mice, with paw weight and surface area placement correlating to paw licking and biting. Optimization of adjustable threshold parameters allowed for reduced coefficients of variability and increased duration of validated data.
Conclusions: The ADWB assay provides an efficient and unbiased measure of chemical-induced hyperacute hypersensitivity in mice. ADWB detection parameters influence amount of validated data and variability, a consideration for data analysis in future studies.

RÉSUMÉ

Contexte: Les méthodes standard d'évaluation de la douleur chez les rongeurs dépendent souvent de l'observateur, ce qui peut fausser les résultats. La mise en charge dynamique avancée offre une approche indépendante de l'observateur qui peut fournir des données objectives et fiables dans la recherche préclinique sur la douleur.
Objectifs: L'objectif de cette étude était de caractériser l'utilisation de la mise en charge dynamique avancée dans l'évaluation des réponses murines à l'hypersensibilité hyperaiguë induite par l'isothiocyanate d'allyle et de répertorier les meilleures pratiques d'utilisation de l'appareil.
Méthodes: Des souris C57BL/6J mâles ont reçu des injections intraplantaires de solution saline ou de solution d'isothiocyanate d'allyle à 0,1 % et ont été évaluées à l'aide du système de mise en charge dynamique avancée; les durées simultanées de léchage et de morsure des pattes, dépendantes de l'observateur, ont été mesurées. Les données obtenues par la mise en charge dynamique avancée ont été analysées à l'aide du logiciel propriétaire de Bioseb et corrélées aux résultats dépendants de l'observateur, avec des paramètres évalués pour optimiser les données collectées.
Résultats: L'essai réalisé à l'aide de la mise en charge dynamique avancée a détecté des changements de poids et de distribution de surface liés à la douleur chez les souris traitées à l'isothiocyanate d'allyle, le poids des pattes et le placement de la surface étant corrélés au léchage et à la morsure des pattes. L'optimisation des paramètres de seuil ajustables a permis de réduire les coefficients de variabilité et d'augmenter la durée des données validées.
Conclusion: L'essai réalisé à l'aide de la mise en charge dynamique avancée fournit une mesure efficace et impartiale de l'hypersensibilité hyperaiguë induite par les produits chimiques chez la souris. Les paramètres de détection du système de mise en charge dynamique avancée influencent la quantité de données validées et la variabilité, ce qui doit être pris en compte pour l'analyse des données dans les études futures.


Introduction

Pain is a complex and subjective experience that affects one's physical and psychological well-being.[1,2] Clinically relevant pain is often spontaneous[3] or "nonreflexive"[4,5] and difficult to treat given a lack of effective, safe therapeutic options. Although new therapeutic targets are identified, the translational success of conventional preclinical animal models has been inadequate.[6] Because pain is a multidimensional, perceptive experience, behavioral "pain" outcomes in mice, such as facial grimacing,[7] paw licking and biting,[8] or paw withdrawal latencies,[9] have traditionally been inferred from observed human-defined behaviors.[10,11] Such traditional measures are limited by their observer dependency, which introduces the potential for subjective bias and potentially unpredictable clinical translation.[12] Though behaviors of grimacing and paw grooming can reflect affective, motivational responses to an injection of a nociceptive stimulus,[7,13] withdrawing a paw is a reflexive response involving different cortical centers[14] that is often not representative of that observed in the clinic.[5,15] Additionally, observer-dependent tests often require extensive experimenter–animal interaction, which may further affect murine behavior and introduce variability.[16–18] For instance, though the static weight-bearing incapacitance test can provide objective weight measurements,[19] it involves an unnatural restraint that may stress the animal. There is therefore a need for established observer-independent strategies to assess pain responses in freely moving animals over time.

Temporal, quantitative, and objective measures of altered behaviors in unrestricted rodents can help advance the assessment of preclinical pain models and potential analgesics. Various groups are developing new automated systems,[20,21] including the use of high-speed cameras,[22] marker-less pose estimation,[23] deep neural networks,[24] and other measures to assess hyperacute (minutes) and acute (hours) pain.[25,26] The voluntary wheel-running assay,[27] for example, uses automated recording of total distances traveled by mice in the absence of an experimenter in the room; analgesics and anti-inflammatory agents have been found to increase distance traveled during pain states. The recently developed Advanced Dynamic Weight Bearing (ADWB) system measures paw placement and weight using automated floor sensors with a camera to record real-time animal activity; proprietary software analyzes these data using adjustable detection parameter settings (i.e., thresholds for weight, surface area, and number of frames). The ADWB allows for (1) the animal to freely explore its environment, facilitating the study of behavioral responses to nonreflexive mechanical hypersensitivity,[28] and (2) the objective measurement of both paw weight and surface area, eliminating bias in the data collection process. To date, this tool has been used to study a range of pain conditions (e.g., postoperative pain,[29,30] cancer-induced bone pain,[31] inflammatory hyperalgesia,[32] neuropathic pain,[33,34] and osteo- and inflammatory arthritis)[35,36] as well as neurological diseases including vestibulopathy[37] and multiple sclerosis.[38]

Though some have suggested that dynamic weight bearing can be used as a measure of neuropathic[33,34] or spontaneous pain-like behaviors,[30–32] there have been contradictory findings with the use of weight bearing as a measure of chronic pain. For instance, there are conflicting views as to the utility of weight bearing as a measure of pain after specific chronic injury models.[39,40] Such discrepancies may be due to how animal behavioral responses are interpreted using various tests. For example, some studies have used the ADWB system to measure changes in *static* weight bearing[41] and the CatWalk system for *dynamic* gait alterations.[39,41,42] However, unrestrained animals are not stationary in the ADWB chamber, allowing for dynamic weight bearing to be assessed. Furthermore, the CatWalk system is reported to have limited detection sensitivity for weight bearing.[43] Thus, a consensus in the field is warranted. Nevertheless, ADWB provides a tool to assess changes in paw weight bearing in unrestrained rodents in an automated fashion, filling a gap left by observer-dependent pain assays by revealing changes in intensity objectively.

Because the ADWB system detects altered weight bearing across limbs, we hypothesized that it may be useful for indirectly measuring mechanical allodynia, a painful sensation characterized as hypersensitivity to non-noxious stimuli, such as light touch.[44,45] Though mechanisms underlying mechanical allodynia are believed to be distinct from those facilitating affective, motivational pain, it is an important aspect of chronic pain that can be measured in hyperacute and acute contexts.[44,45] Here, we induced hyperacute pain via an intraplantar injection of the pungent chemical irritant allyl isothiocyanate (AITC, or "mustard oil"), which activates the transient receptor potential (TRP)-ankyrin-1 (TRPA1) channel.[46,47] AITC is used to model noxious chemical-induced pain sensitization,[48] neurogenic inflammation,[49] and mechanical hypersensitivity.[50–52] Though the TRPV1 agonist capsaicin was previously used to study chronic effects in dynamic weight bearing, we sought to characterize for
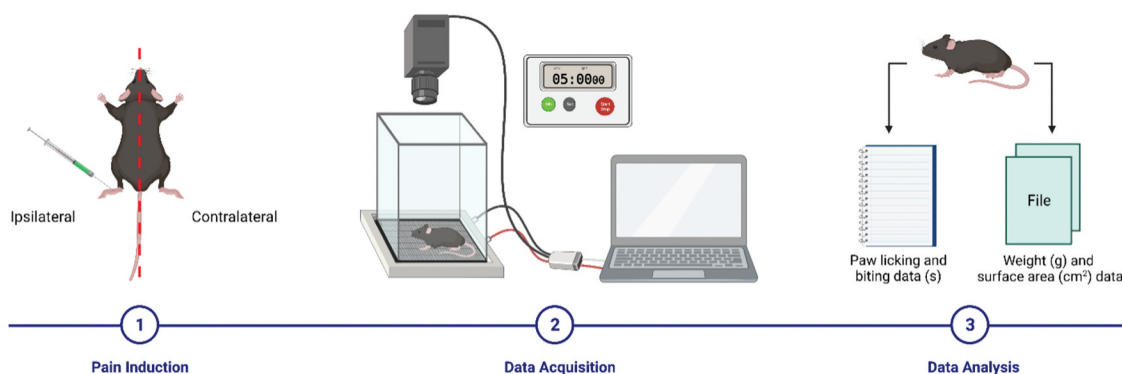
Figure 1. Schematic diagram of experimental protocol. Male C57BL/6J mice received a 20-μL intraplantar injection of 0.1% AITC or saline (vehicle) into the rear left (ipsilateral) footpad. Observer-dependent behavioral responses were manually recorded using a stopwatch (measured as time [seconds] spent licking and biting the injected paw). Observer-independent analysis was conducted using the advanced dynamic weight bearing system (pictured in [2]) to assess weight and surface area of each paw on the sensor pad.

the first time the utility of ADWB as an observer-independent assessment of behavioral responses to AITC-induced hypersensitivity in the footpad in the hyperacute phase (minutes after onset of pain) and to define the optimal system parameters for data analysis.

Materials and Methods

Animals

We assessed changes in weight bearing of mice injected with AITC in the ipsilateral hind paw as a strategy to observe pain-like outcomes in an observer-independent manner (Figure 1).

Experiments were carried out using 7- to 10-week-old (22–36 g) male C57BL/6J mice ($n = 40$). Mice were housed in groups of two to four per ventilated cage in a temperature-controlled room (21°C ± 1°C) on a 12-h light/dark cycle. Animals were fed a standard rodent diet and water was provided ad libitum. Experimental protocols for animal use and care followed those outlined and approved by Queen's University Animal Care Committee (protocol #2019-1936), in accordance with the Canadian Council on Animal Care guidelines. The data presented here are from two independent experiments ($n = 8$–10/study) conducted at different times of day.

Pain Induction with Allyl Isothiocyanate

AITC was resuspended in 100% ethanol to produce a 10% stock solution and diluted to obtain a final concentration of 0.1% in 0.9% NaCl. Unanesthetized mice, restrained using a soft microfiber cloth, received a single 20-μL intraplantar injection into the rear left footpad with either 0.1% AITC solution or saline with 1% ethanol (equivalent to ethanol concentration in AITC solution) as a sham-injected control.

Data Acquisition

Advanced Dynamic Weight Bearing

Data were collected as previously described, with slight modifications.[38] The ADWB apparatus and software (v.1.4.2.98, Bioseb, Vitrolles, France) was used to measure nonreflexive pain. Its chamber consists of a transparent plexiglass cage (11 cm wide × 11 cm long × 20 cm high) equipped with a pressure-sensitive floor and an overhead high-resolution camera. Prior to data acquisition, the ADWB sensor was tared and calibrated, with ambient room lighting kept at a minimum. Mice were habituated to the ADWB testing assay for at least 5 mins 1 day prior to experiments. On the day of testing, mice received an intraplantar injection and were immediately placed into the ADWB chamber; only one mouse was placed in the chamber at a time. The chamber was cleaned with a 100% ethanol wipe before and after each observation period. The floor sensors automatically recorded the weight and surface area distributed between the paws and other body parts (e.g., tail) during the 5-min data acquisition period.

Observer-dependent Measurement

Cumulative time (in seconds) spent licking and biting the rear ipsilateral paw was recorded by an experimenter by stopwatch for a period of 5 mins, concurrent with ADWB data acquisition. The time between when the rear ipsilateral paw made and broke contact with the mouth was recorded. Rear paw or limb lifting directed toward the mouth was not recorded.

Data Analysis

ADWB Scoring Analysis

By design, experimenters were blinded to the weight and surface area measurements of each zone during manual scoring, confining the subjective interpretation to simply determining the orientation of the animal using the frames of the video as reference. Briefly, ADWB software displays collected data on a timeline on which sections or "segments" of consecutive data frames that meet the criteria set out by the detection parameters are included in results, whereas invalid sections—possibly a result of the animal moving too fast or light reflections—are excluded from the results. To ensure that each successive frame within a given segment was correctly scored, data were analyzed frame by frame along the timeline (rather than segment by segment) until all validated data were assessed; a minimum of two images or frames were collected for all data analyzed. Sensor activation from the tail, abdomen, genitals, or head was manually designated and included in the results as "other." Likewise, a "grouped" designation defined instances where the front paws were too close in proximity to be distinguished. Pseudo contacts (e.g., feces) were designated as "ignored" and excluded from the experiment. If the animal's position on the video did not correspond with the labeling of pixels on the sensor feed, the experimenter manually adjusted the contact labeling.

Exclusion Criteria

Validated data, defined as the time during which an animal's position is adequately stable to allow for robust sensor detection and software recognition, were automatically calculated by the ADWB system and quantified as time (in seconds). Mice with sessions containing <30 s of validated data were excluded from all analyses. As such, four mice were excluded (two mice each from the saline and AITC groups).

Statistical Analysis

All statistical analyses were carried out using SigmaPlot (v.11, Systat Software, Palo Alto, USA). Independent t tests and analysis of variance (ANOVA) were used to compare observer-dependent (paw licking) and observer-independent (ADWB) data, with significance thresholds set at $P < 0.05$, using the post hoc Tukey test and Holm-Sídak multiple comparisons test. Pearson correlations were used to assess the relationships between observer-dependent and -independent data.

Results

ADWB Detection Parameters Impact Variability and Amount of Validated Data but Not Calculated Weight

The ADWB software uses algorithms influenced by adjustable settings known as the detection parameters, which include weight detection and surface area thresholds. Although a few studies have described the detection parameter settings used, pinpointing a potential source of variation on data collected from these settings has not been investigated to date. To investigate whether these parameters influence the coefficient of variability and duration of validated data, we adjusted the standard detection parameters of files from control groups. Standard ADWB detection parameters (termed "default") are set to 0.8 and 1.0 g for the low/high weight thresholds, two responsive load cells for surface threshold, and three frames for minimum number of frames analyzed. We adjusted these parameters (termed "adjusted") to 0.3/0.5 g for the low and high thresholds, as used in a previous publication from our group. Comparison of these two parameter settings revealed significantly increased time of validated data per acquisition period (Figure 2a, $P = 0.008$) and significantly reduced coefficient of variability (standard deviation/ mean; Figure 2b, $P < 0.001$) in the "adjusted" group compared to "default." Adjusting the parameters did not significantly impact left/right (L/R) ratios for weight or surface area (Figure 2c, $P = 0.487$). We therefore used the "adjusted" parameter settings for all subsequent analyses as a result of the increased time of validated data collected and reduced variability. These results constitute our second objective, to help inform best practices for use of the ADWB device.

ADWB Analysis Detects Changes in Weight and Surface Area Distribution

Hyperacute mechanical hypersensitivity was induced with a single injection of 20 μL of 0.1% AITC into the rear left paw of a male mouse, with changes in weight bearing (g) and surface area (mm^2) quantified using the ADWB apparatus. Significant reductions in surface area and weight distribution were detected in the ipsilateral fore- and hindpaws of mice injected with AITC, relative to contralateral paws (Figure 3a, $P = .004$; Figure 3b–d, $P < 0.001$). Importantly, no such differences were observed in surface area (Figure 3b, $P = 0.097$; Figure 3d, $P = 0.767$) or weight distribution (Figure 3a, $P = 0.070$; Figure 3c, $P = 0.841$) in the saline-treated (vehicle control) group. Postural changes were also observed in AITC-treated mice as reflected by the significantly increased weight and surface area born on the
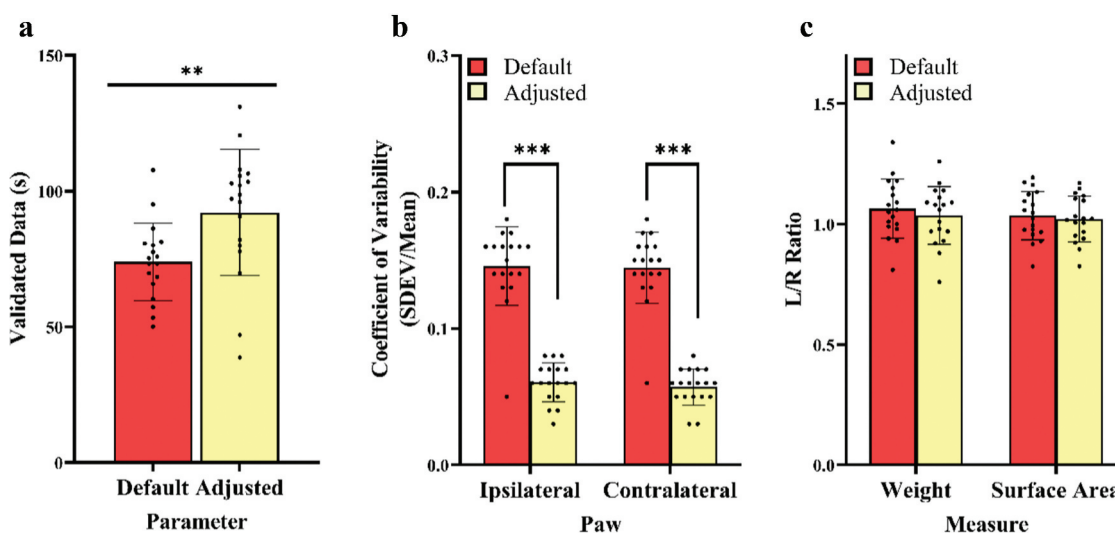
Figure 2. Coefficient of variability is affected by amount of validated data collected but not left/right (L/R) ratios. Male C57BL/6J (n = 18) mice received an intraplantar saline injection and were subjected to a 5-min advanced dynamic weight bearing data acquisition period. Resulting data were scored twice using default (low, high weight thresholds: 0.8 g, 1.0 g; minimum number of images: two frames; surface threshold: three units) and adjusted (low, high weight thresholds: 0.3 g, 0.5 g; minimum number of images: two frames; surface threshold: two units) parameters. The (a) mean amount of validated data (time, seconds) per animal and (b) coefficient of variability were altered after adjusting parameters. (c) L/R ratios did not differ between parameter sets used. All data were analyzed using an independent *t* test with post hoc Tukey's test comparing default vs. adjusted and presented as mean ± SD. Data points = individual mice. **$P \leq 0.01$. ***$P \leq 0.001$.

grouped front paws (Figure 3e, $P = 0.006$; Figure 3f, $P = 0.003$) and in other body regions (i.e., tail and abdomen; Figure 3g,h, $P < 0.001$) compared to vehicle controls. The L/R ratio of paw weight and surface area was decreased in AITC-treated mice compared to control groups (Figure 4a,b, $P < 0.001$). Moreover, the ratio between rear and front (Re/Fr) paw weight and surface area was decreased in AITC-treated mice compared to controls (Figure 4c,d, $P < 0.001$). Together, these findings indicate that the AITC group shifted weight toward the front right (contralateral) supporting limbs, possibly in an attempt to maintain balance while relieving pressure on the injected paw (Figure 4). This is further supported by the moderate negative correlation between L/R weight ratios and paw licking and biting time, wherein AITC-treated mice spent more time licking and biting their injected paw, along with reduced weight bearing (Figure 5a, $R = -0.680$, $P = 0.00192$). This correlation was also observed for L/R surface area ratios (Figure 5a, $R = -0.480$, $P = 0.0437$), possibly because applied surface area of an individual or group of paws is independent of the total applied surface area and can vary greatly, whereas total body weight remains constant despite changes in weight distribution. Similarly, the Re/Fr surface area and weight ratios did not correlate with paw licking and biting time for either the weight (Figure 5b, $R = -0.197$, $P = 0.433$) or surface area (Figure 5b, $R = -0.127$, $P = 0.616$); this may be a result of mice redistributing their weight and surface area to the

"other" regions, which, unlike the front paws, are not accounted for in the Re/Fr computation. Sample size calculations using this data set have helped determine that $n = 9$ animals are necessary for future studies to reach power of 0.90 with $\alpha = 0.05$ (independent groups, two-tailed *t* test).

ADWB as an Observer-Independent Measure of Pain Over Time

The ADWB system collects data continuously, allowing the investigation of responses to AITC over time. We recorded and compared findings from continuous 5-min periods; analysis of the confidence intervals of changes in percentage weight and surface area found the greatest reduction in response during the first minute after injection relative to control injections; the nocifensive response in AITC-injected mice is apparent for at least 5 mins postinjection (Figure 6a,b). The mean weight placed on the rear ipsilateral paw was ~20% (with 50% reflecting normal paw distribution) during the first minute in AITC-injected mice before leveling to ~40% of total rear paw weight. A comparison between the first minute after injection and the following 4 mins revealed significantly less weight and surface area born on the ipsilateral paw during the first minute (Figure 6c, $P < 0.01$; Figure 6d,
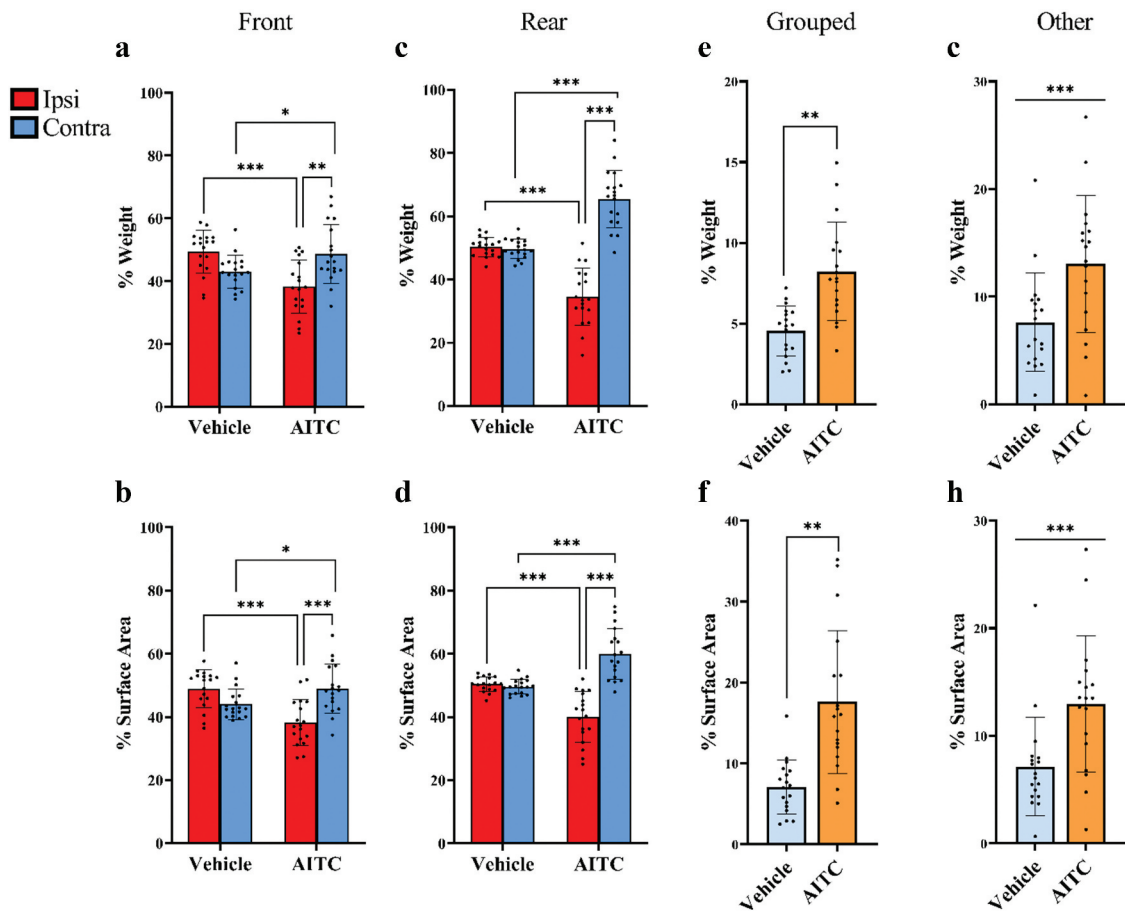
Figure 3. Objective measures of paw weight and surface area placement show changes across front/rear and ipsilateral/contralateral paws. Male C57BL/6J mice each received a single intraplantar injection of AITC or saline (vehicle control); pain responses were assessed by ADWB for 5 mins. AITC-treated mice redistributed the weight (g) and surface area (mm^2) of their front (a and b, respectively) and rear (c and d, respectively) ipsilateral ("ipsi") paws to their contralateral ("contra") paws and increased weight and surface area born on their grouped front paws (e, f) and other body parts (g, h). Weight and surface area are expressed as a percentage of total weight or surface area for each set of front or rear paws, grouped front paws ("grouped"), or body ("other"). All data str presented as mean ± SD and (a)–(d) were analyzed using a two-way ANOVA and post hoc Tukey's test and (e)–(h) independent t tests. Data points = individual mice. *$P \leq 0.05$. **$P \leq 0.01$. ***$P \leq 0.001$.
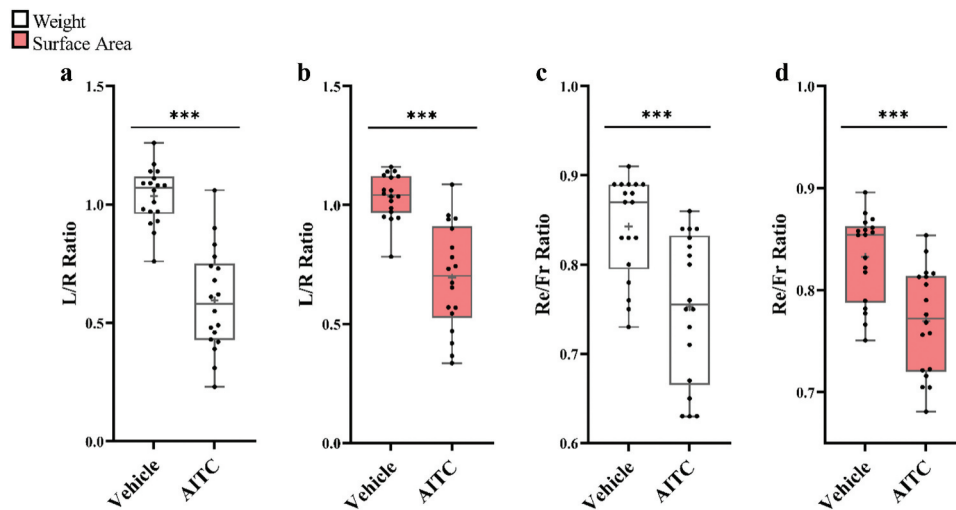
Figure 4. Left/right and rear/front ratios for both weight and surface area placement of footpads are altered in response to intraplantar AITC injection. (a), (b) The weight and surface area ratio of ipsilateral (left; "L") to contralateral (right; "R") paws was decreased in AITC-treated versus vehicle controls. (c), (d) The ratio of weight and surface area between rear and front paws (Re/Fr) was decreased in AITC-treated versus vehicle controls. Data were analyzed by independent t test and presented as mean ± SD. Data points = individual mice. ***$P \leq 0.001$.
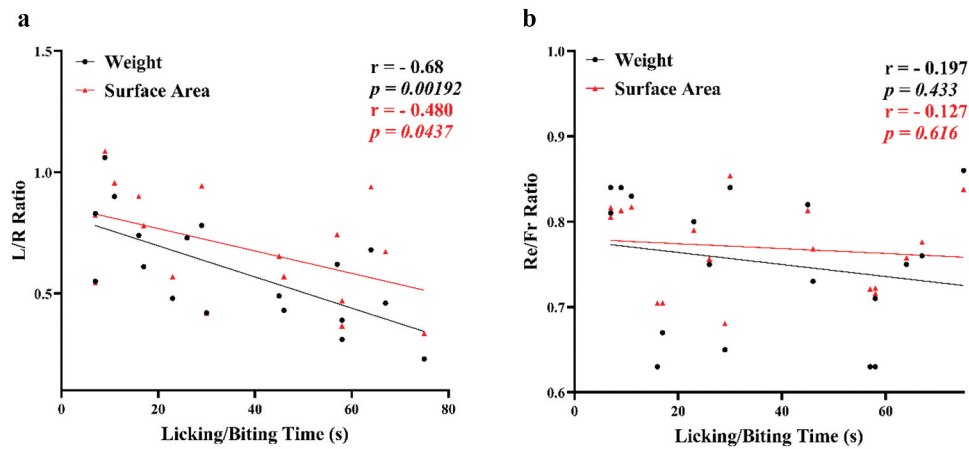
Figure 5. Observer-independent detection of weight-bearing patterns correlated to observer-dependent paw licking and biting times. Weight ratios (black lines) for (a) L/R ($R = -0.680$, $P = 0.00192$) and (b) Re/Fr ($R = -0.197$, $P = 0.433$) across all animals studied revealed a negative correlation to paw licking and biting time (measured in seconds). No significant relationship exists for surface area across both L/R and Re/Fr ratios (red lines). (a) $R = -0.480$, $P = 0.0437$; (b) $R = -0.127$, $P = 0.616$. Correlations were assessed using a Pearson's correlation test. Data points = individual mice.
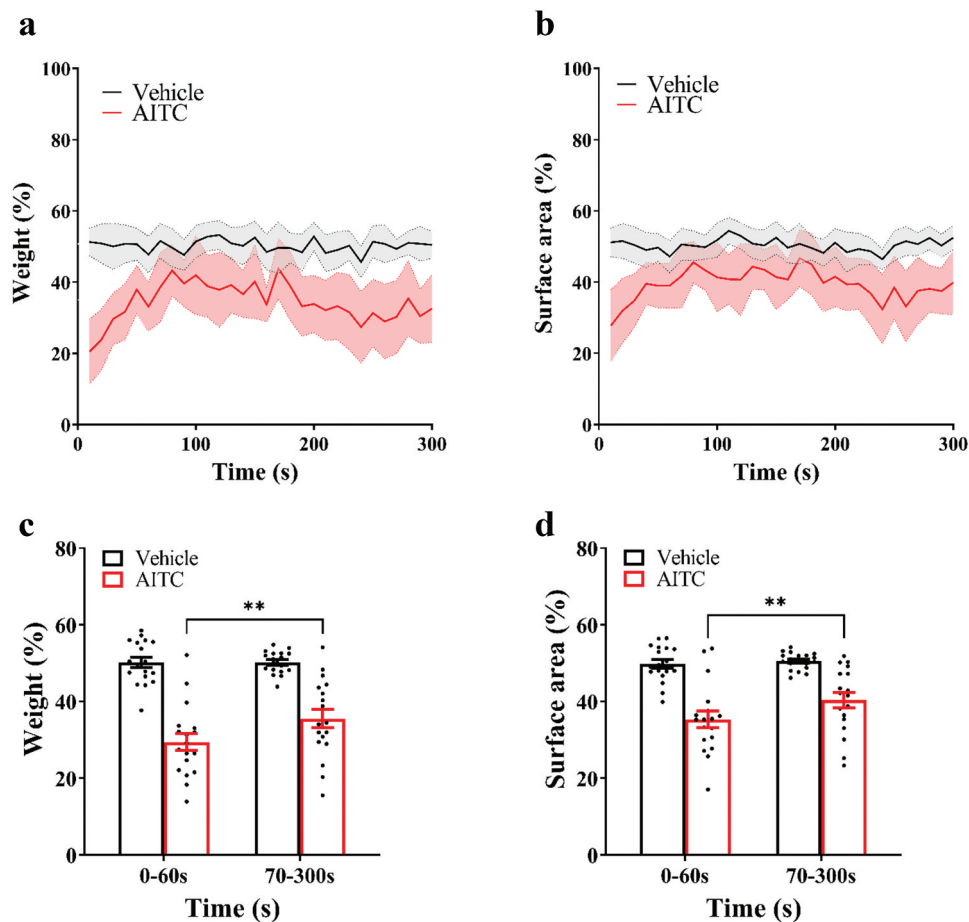
Figure 6. Responses to AITC-induced acute peripheral nociceptive pain over time. (a), (b) Confidence intervals (95%) show reduced (a) weight bearing and (b) surface area placed on the ipsilateral paw during the 5-min postinjection period in AITC-injected mice relative to vehicle-treated control mice. Lines represent the group mean for each time point. (c), (d) Time comparison of 5-min observation period showing significantly reduced (c) weight and (d) surface area bearings during the first minute compared to the remaining 4 mins. Data are presented as mean ± SD and were analyzed using a two-way ANOVA with post hoc Sidak's test. Data points = individual mice. **$P \leq 0.01$.

$P < 0.01$). These data constitute the first objective, quantification of mouse responses to an intraplantar injection of AITC.

Discussion

To our knowledge, this is the first study to report an in-depth analysis of ADWB as a tool to study hyperacute (first 5 mins) murine behavioral pain responses and the first using intraplantar injection of the TRPA1 agonist AITC. We show that the ADWB system can successfully detect differences in paw weight and surface area distributions between AITC- and saline-treated male C57BL/6J mice over 5 min postinjection and identify key parameters to capture these effects with minimal variability and maximal data acquired for analysis. Though many studies have relied on behavioral tests that concentrate on a snapshot of the stimulated body part, ADWB allows for the assessment of dynamic behaviors at the system level through automatically computed ratios between the left/right and rear/front paws. These measurements revealed AITC-treated mice distributed their weight and surface area to favor the uninjured contralateral paw along with increased weight bearing on the front paws, indicative of pain localized to the rear ipsilateral paw that was injected with AITC; these results were not detected in the control saline-treated group. Evaluation of the relationship between ADWB and our observer-dependent assay revealed that distribution patterns of paw weight, but not surface area, were consistent with patterns of paw licking and biting. Taken together, our results support the use of ADWB as an alternative to conventional observer-dependent measures of pain in rodent experiments.

To date, few pain studies using ADWB have provided sufficient detail for reliable reproduction of experimental paradigms across laboratories.[34,36,53] Further, though some publications using the ADWB system have focused their analysis on weight and surface area measurements,[32] findings can be reported based on validated time as well, which other studies have done.[54] Given that the contribution of these features to reproducible data collection has not been addressed in detail, we sought to explore the impact of parameter settings on data output. Though we found no significant changes in weight and surface area measurements between default and adjusted parameters, we do show that adjustment of the high and low weight thresholds and "minimum number of images" parameters impacted the amount of validated data and variability of the results acquired using the software. These differences highlight the importance of clarifying whether experimental subjects

were analyzed with the same or different parameters, because we showed a significant increase in validated time when we adjusted the standard parameters to the ones previously described for mice.[38] Thus, future studies using ADWB should adjust these parameters for optimal data collection and report the parameters used to improve data reproduction, interpretation, and comparison across laboratories.

Chemical activation of TRPA1-expressing nociceptors produces an intense burning pain sensation,[55] with TRPA1-knockout mice lacking sensitivity to AITC.[56,57] This pain induction model has been previously shown, using observer-dependent assays, to induce peak acute nociception within the first 3 mins postinjection, manifesting itself with increased licking of the injected footpad[58]; mechanical hypersensitivity can also persist after injection for over an hour.[59] This was consistent with our findings that showed consistently decreased ipsilateral paw weight in the AITC group compared to controls over 5 mins after injection, with the greatest effect evident during the first minute after injection (see Figure 6). The ability for continual measurement of nociception provides an added benefit of ADWB to study the onset and pattern of hypersensitivity in animal models. However, some exceptions were observed in our data set where particular mice had clearly reduced injured paw weight and surface area placement despite spending little to no time licking and biting the affected paw. These discrepancies may be the result of masking behaviors, a common survival tactic in injured prey species to avoid attracting predators.[60,61]

Though the current study represents the first use of ADWB for examination of pain behaviors in response to AITC at hyperacute time periods (i.e., minutes), ADWB was previously employed in models of inflammatory arthritis induced by intra-articular injection of the potent TRPV1 agonist capsaicin,[36,53] as well as the chemical irritants complete Freund's adjuvant[21,36,62] and carrageenan,[63] to study acute (hours) to chronic (days) stages of pain behaviors. An understudied yet clinically relevant aspect of chronic pain is the spontaneous, nonevoked response,[15] for which there is currently disagreement on the behaviors that reflect this outcome. Though the validity of weight bearing as a measure of chronic neuropathic pain requires further study, there is growing evidence of its usefulness as a measure of chemical-induced and inflammatory pain.[16,32,36,62] Thus, a more critical examination of specific behaviors in real time as a potential measure of spontaneous pain is necessary.

Attempts to elucidate underlying mechanisms of pain modalities have revealed complex interactions between systems. For example, administration of a subanesthetic

dose of ketamine abolished affective (paw licking) but not reflexive (paw flicking) observer-dependent behaviors,[64] suggesting a mechanistic distinction between distinct pain modalities. Whether reduced weight bearing can reflect changes in motivation-directed behaviors (i.e., rearing[65]) related to clinically relevant spontaneous pain is not entirely understood and was not evaluated in this proof-of-concept study. However, we provide evidence for use of the observer-independent ADWB system in the hyperacute (minutes) phase to detect changes in weight bearing in response to chemical-induced mechanical hypersensitivity.

Given the limitations of observer-dependent assays, several groups have begun to develop new technologies to assess pain behaviors in preclinical animal models. These approaches include increasing camera resolution of videographic techniques to reduce experimenter interference and improve ease of manual scoring.[66–68] These new technologies allow more experimenters to identify more precise observations in an automated, objective manner. Moreover, these technologies have the potential to simplify data comparison across studies and laboratories.[66] For instance, results from freely moving animals captured by machine-detected, automatic systems can be reported as ethograms, depositing records of animal behaviors into databases.[67] Recently, Bohnslav and colleagues developed DeepEthogram,[67] a Python program capable of learning to automatically detect and label distinct behaviors, allowing for minimal user input. However, limitations may still arise in these new videographic technologies. For instance, recording mouse somatosensory behaviors, coupled with manual behavioral mapping, statistical modeling, and machine learning, confers the advantage of allowing for more objective "pain scales,"[22,67,68] yet human error cannot be entirely excluded because this software requires a user for the initial training of the machine to detect the behaviors of interest present in each video frame. Though ADWB is similarly not exempt from this limitation, human error or bias in manual scoring can be lessened through (1) using frame-by-frame analysis instead of a segment-by-segment navigation approach; (2) minimizing room lighting to reduce glare on the sensors, which can interfere with video capture; and (3) adjusting detection parameters to optimized, experiment-dependent data capture settings.

The present study using ADWB is not without limitations. Though ADWB was able to detect differences in weight bearing, the integration of these data with the video feed captured simultaneously could be improved, allowing for a more automated and less time-consuming analysis of data. Manual scoring in a frame-by-frame manner is labor intensive and time-consuming, and the lack of a side-view camera with the device introduces the potential for error due to restrictive viewing angles. Further, measuring paw elevation time would be an ideal parameter to reflect pain behavioral responses; however, this is not possible due to the method through which data is collected by this system. Moreover, $n = 4$ samples were omitted from further analysis due to software-related technical issues, which represents a large error rate (10%) for this type of analysis. Though only male mice were used, our study provides a proof-of-concept methodology for future studies using ADWB. Additional work will be necessary to further demonstrate the utility of ADWB in detecting and evaluating analgesic effects of currently available therapeutics[30] and/or testing preclinical TRPA1 inhibitors.[69] However, others have shown that this system can detect changes in response to drugs such as diclofenac, indomethacin, and celecoxib in various models of acute and chronic pain.[22,28,30,70]

The work presented here was driven by a need for reliable data collected from automated, observer-independent tools used to assess behavioral responses to pain in rodent models. Our findings further validate the use of ADWB as an observer-independent tool to reveal postural shifts in response to peripheral nociceptive pain, and now show its usefulness to capture hyperacute responses. We also suggest that future pain studies using ADWB report the parameters used for optimal reproducibility and consistency, which will ultimately lead to better identification of treatment targets and accelerated therapy development.

Disclosure Statement

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Reb Statement

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