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Automated assessment of pain in rats using a voluntarily accessed static weight-bearing test

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HIGHLIGHTS

- We developed a novel device (VASIC) for automated weight-bearing pain measurement.
- The VASIC device is compatible with conventional hind-limb injury pain models.
- The device allows hands-free high-through put collection of unbiased behavioral data.

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ABSTRACT

The weight-bearing test is one method to assess pain in rodent animal models; however, the acceptance of this convenient method is limited by the low throughput data acquisition and necessity of confining the rodents to a small chamber.

New methods: We developed novel data acquisition hardware and software, data analysis software, and a conditioning protocol for an automated high throughput static weight-bearing assessment of pain. With this device, the rats voluntarily enter the weighing chamber, precluding the necessity to restrain the animals and thereby removing the potential stress-induced confounds as well as operator selection bias during data collection. We name this device the Voluntarily Accessed Static Incapacitance Chamber (VASIC).

Results: Control rats subjected to the VASIC device provided hundreds of weight-bearing data points in a single behavioral assay. Chronic constriction injury (CCI) surgery and paw pad injection of complete Freund's adjuvant (CFA) or carrageenan in rats generated hundreds of weight-bearing data during a 30 minute recording session. Rats subjected to CCI, CFA, or carrageenan demonstrated the expected bias in weight distribution favoring the un-operated leg, and the analgesic effect of *i.p.* morphine was demonstrated. In comparison with existing methods, brief water restriction encouraged the rats to enter the weighing chamber to access water, and an infrared detector confirmed the rat position with feet properly positioned on the footplates, triggering data collection. This allowed hands-off measurement of weight distribution data reducing operator selection bias.

Conclusion: The VASIC device should enhance the hands-free parallel collection of unbiased weight-bearing data in a high throughput manner, allowing further testing of this behavioral measure as an effective assessment of pain in rodents.

1. Introduction

Pain affects more Americans than diabetes, heart disease, and cancer combined and is a leading cause of disability and a major contributor to health care costs [1]. A clear medical need exists for the discovery of more effective, better-tolerated, and safer analgesics [2,3]. Thus, extensive research and development efforts in both academia and industry have been directed towards the discovery of novel analgesics. However, despite significant effort, there has been a lack of breakthrough discoveries during the past half century [4,5]. Of the potential drugs that go through the development pipeline, the leading cause of failure is lack of efficacy in humans. This number is estimated to be the cause of about 30% of all failures in the very costly clinical phase of drug development [6]. Tetreault [5] points out that the weak preclinical prediction of clinical efficacy may be partially a result of limitations of pain assessment in animal models.

The dominant paradigm in analgesic drug development has relied heavily on behavioral pharmacology in laboratory animals. Since non-human animals cannot self-report, acute behaviors in response to noxious stimuli were used as an index to gauge pain in experimental animal models. Traditional preclinical assessment of pain in animals has relied

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on measuring the reflexive withdrawal of a limb to physical stimuli, including punctate tactile stimuli, deep pressure, heat or cold [7–9]. However, there has been ongoing discussion and criticism in the field that these traditional assays may be insufficient to fully encompass the range of clinical pain and do not test the nature of spontaneous pain. Traditional assays such as the von Frey assay and plantar test rely on a behavior paradigm which requires an acute stimulation to induce an evoked readout of reflexive responses. These assays are prone to confounding factors due to forcefully stimulating the animal for a reflex response and the potential observer bias due to often unintentional incomplete blinding of the operator [10]. The issue of whether we can rely on these tests to have a predictive validity for a pre-clinical pain study is in question since they: 1) can be evoked without supraspinal processing as in spinalized animals and the reflexes are not necessarily cerebral-mediated pain responses (for tail-flick reflex), 2) are affected by surgical damage to axons of motor neurons, and 3) lack predictive value [11–15]. Noxious stimulus-evoked reflexive responses most likely do not involve cognitive and emotional aspects of clinical pain, although these dimensions of pain are not directly assessed by the traditional behavioral assays. While the field still lacks clearly defined and well-accepted behavioral paradigms most appropriate for assessing pain in animal models, there have been new attempts to incorporate the affective component of pain with various behavioral paradigms (reviewed in Li [16]).

A static weight-bearing device using a dual-channel weighing apparatus was developed and historically used as a clinical tool in orthopedics as an indicator of pain [17], as well as to monitor changes in post-surgical recovery and gait [18]. The use of the weight-bearing test to assess arthritic pain in rodents was first introduced by Schöt et al. [19] and subsequently utilized to assess pain in various experimental models including neuropathic, inflammatory, and cancer pain [20–22]. The advantage of the static weight-bearing test is that it is allegedly an objective measure of pain that does not involve artificial external stimuli and is applicable to a large spectrum of animal pain models in hind limb. Also, unlike evoked pain response paradigms, the weight-bearing distribution has been often included in the repertoire of behaviors that assess spontaneous pain as well as guarding behavior of the site of pain or injury [14]. However, the conventional static weight-bearing test requires restraining the animals in a small cage which confines them to an unnatural posture during the period of measurement. Two issues arise from conventional behavior assays such as the incapacitance meter. Such restriction of movement against the animals' desire during the test may obscure the data by either invoking acute stress-induced analgesia [23,24] or chronic stress-induced hyperalgesia [25,26]. Also, repeated acute restraint of rodents has been shown to induce modulation of conventional pain readouts [27–29]. As an alternate solution, a dynamic weight-bearing (DWB) device that reduces the potential restraint-stress was introduced and validated [5,30] for its compatibility with conventional pain models. Although DWB is a more sensitive free-moving behavior system requiring less handling, a major disadvantage of the DWB device is that the analysis requires labor intensive manual integration of the multiple video and sensor data [30].

We introduce a novel automated, free-moving, and high-throughput weight-bearing device as a convenient method for assessing pain in rodents. Our device combines the basic concept of the static weight-bearing test with a novel chamber modification to the measurement apparatus to allow a simple behavioral task and an appropriation of a thirst satiation reward-driven voluntary behavior of the animals to measure its own weight without an operator. As such, the present paradigm incorporates an affective component to the pain assessment since the rats must assess the reward of satiating thirst vs. the discomfort of pain. Therefore, our device circumvents the limitation of conventional behavioral assays requiring a restraint or extensive handling of animals while allowing the experimenter to obtain hundreds of consistent and independently replicable unbiased weight distribution data. The implemented hardware, software, and behavioral paradigm allow rapid accumulation of unbiased weight-bearing measures with minimal user intervention suited for a large-scale automated pain assessment in rodents required for analgesic development.

2. Materials and methods

2.1. Voluntarily Accessed Static Incapacitance Chamber (VASIC)

The analog weight information obtained from the load cells updated at a 100 millisecond interval was converted to digital data by an on-board analog-to-digital converter. The local microprocessor averaged the weight data over a user-defined time interval, and the averaged weight data was transmitted to the host computer via a Windows simulated serial port on a USB port. The detection of correct animal position within the smaller inside chamber was determined by the infrared (IR) beam breaker detector positioned below the water source that can only be reached when the animal positions itself on the weighing platforms. Once captured by the host computer, the weight data for right and left sides, along with a time stamp, were collated as a text file and saved. An analysis software developed in Excel (Microsoft, Seattle, WA) read the text data, applied user-selected data filters, and wrote out the processed data onto an output column of the spreadsheet. Figures were made, in Excel, from the output text files. Fig. 1 shows photographs of the animal voluntarily positioned in the behavioral chamber to access water as well as a block diagram of the relevant components of the electronic circuit. The current VASIC model allows detection sensitivity of 0.2 g up to 800 g for each foot pad. The inner chamber and software calibration is designed and optimized to accommodate a rat size ranging from a lower limit of approximately 70 g to an upper limit of 500 g by body mass. A schematic of the device along with the physical dimensions and key hardware components is listed in Supplemental Fig. 1 and Supplemental Table 1. The VASIC device and the data analysis software will be commercially available in the near future. Meanwhile, further details of the hardware design and software can be obtained from the corresponding author.

2.2. Chronic constriction injury rat model

All studies were approved (Protocol M02476) by the local institutional animal care use committee, and all animals were treated in accordance with published NIH standards. Male Sprague Dawley rats weighing 150–200 g, purchased from Harlan Lab (Madison, WI), were used for this study. General anesthesia was induced by delivery of 3–4% isoflurane in oxygen at 3 L/m. Once the animal was unconscious, 1.5% isoflurane in oxygen was introduced at a rate of 2 L/m via a nose cone to maintain anesthesia during the surgery. Upon clipping the hair on the dorsal aspect of the left hind leg, the surgical area was cleaned with 70% ethanol and povidone–iodine scrub solution. An incision of 1.5–2 cm was made dorsal to the pelvis where the biceps femoris and left gluteus superficialis are separated. A small incision was made between the two muscle bellies to expose the sciatic nerve and isolated by blunt dissection with forceps. Using forceps, four loose ligations were made using 4-0 chromic gut suture such that the distance between each knot was less than 1.0 mm apart. The biceps femoris and gluteus superficialis muscle layer and the skin were closed up using a simple interrupted pattern with 5-0 nylon sutures. Animals were assessed daily post-operatively to detect any signs of excessive pain. None of the animals showed behaviors indicative of excessive pain, such as significant appetite change, aggression, or autotomy. In rats receiving morphine sulfate (2 mg/kg), the drug was administered intraperitoneally (*i.p.*) 30 m before the behavioral assessment.

2.3. Complete Freund's adjuvant or carrageenan injection

Inflammatory pain was induced by injecting 150 μL of CFA 50% m/v 1:1 emulsion of 75 μg *Mycobacterium tuberculosis* dry cells (Sigma, St. Louis, MO, USA) in 75 μL phosphate buffered saline inter-dermally or 100 μL of 1% w/v λ-carrageenan (Sigma, St. Louis, MO, USA) in normal saline solution in the left hind paw pad using a 25-gauge needle.