Physiology & Behavior 149 (2015) 229–238

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Assessment of incising ethology in the absence and presence of jaw muscle hyperalgesia in a mouse home cage environment

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HIGHLIGHTS

- A multiaxis force transducer was used to assess incising in a mouse model.
- Sex differences for several incising parameters were identified.
- Incising was composed of five discrete frequencies, not a continuous distribution.
- Female incising was affected more than male incising in a pre-clinical model of pain.
- This technique minimizes experimenter bias and is conducted in the mouse home cage.

ARTICLE INFO

Article history:
Received 27 February 2015
Received in revised form 18 May 2015
Accepted 10 June 2015
Available online 12 June 2015

Keywords:
Central pattern generator
Mastication
Incising
3D force transducer
Pain

ABSTRACT

Introduction: Assessment of oral motor behavior in a mouse is challenging due to the lack of currently available techniques that are non-invasive and allow long-term assessment in a home cage environment. The purpose of this study was to evaluate incising behavior using mouse chow attached to a three-dimensional force transducer that was mounted on the existing home cage. In addition, a persistent hyperalgesia condition was introduced to evaluate the sensitivity of the technique to identify incising behavioral changes.
Methods: Incising activity of CD-1 male and female mice (n = 48) was evaluated over a 24 hour recording session during four baseline and six longitudinal hyperalgesia assessment sessions using custom written software. A pre-clinical persistent pain model was used to induce hyperalgesia in the masseter muscle by repetitive acidic saline injections. Sex and age differences were evaluated for multiple incising variables during both light and dark cycles during baseline and hyperalgesia conditions.
Results: Significant sex differences were found for multiple incising variables but not for age. Discrete incising frequencies were identified in the range of 4.6–10.4 Hz and were reproducibly found in both female and male mice. A significant shift to lower incising frequencies was observed after repetitive acidic saline injections compared to neutral saline injections. This shift to lower frequencies of incising returned to baseline levels after approximately four weeks but was statistically longer in female compared to male mice. Significant differences were also found for chow intake (reduced) and weight change during the hyperalgesia condition. No significant differences were found for total number of incisions or number of incising episodes per day or incising force.
Conclusions: The findings from this study support the use of recording three dimensional incising forces as a sensitive measure of incising behavior. This novel technique allowed the identification of specific incising variables that were differentially affected in female and male mice during a persistent hyperalgesia. The data were collected in the home cage environment with minimal bias such as experimenter interaction. Similar to other dental pain studies, mice were able to maintain normal incising activity levels per day (total incisions, total number of incising episodes) even in the presence of hyperalgesia.

1. Introduction

Oral motor assessment in a rodent model such as mice can provide insight into the effects of genetic disorders, anxiety/stress or medications on the central pattern generator for mastication and feeding behavior. Although food and water intake measures over time can provide some indication of deficiencies or alterations in feeding, these

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http://dx.doi.org/10.1016/j.physbeh.2015.06.015
0031-9384/

measures do not differentiate the impact of reduced satiation (reduced drive), changes in oral motor control (reduced masticatory ability) and reduced overall activity.

Assessment of oral motor behaviors in mice during incising and mastication has been limited by the invasiveness and associated stress of the different recording techniques, the short duration of the recordings and the unique environment outside of the traditional home cage. Multiple techniques have been reported such as mandibular strain gauge recordings [1], electromyographic (EMG) monitoring [2–4] and/or jaw tracking using a magnetic field sensor mounted on the head [5–7]. Many of these techniques are invasive and require surgical implantation of electrodes and tethering the mouse to recording equipment or have telemetry instrumentation implanted. Most recordings from these techniques have been short in duration, lasting from a few minutes to a few hours while eating different foodstuffs such as grains or chow [3,4].

Evaluation of behavior has advanced in recent years using automated computer assessment of video recordings to identify behavior and tracking of activity in the home cage environment. The advantages of these approaches compared to the traditional assessments of behavior during normal and experimentally altered conditions have been discussed by Spruijt et al. and are significant [8]. Evaluation of incising or mastication is especially problematic since small changes in jaw position or bite force cannot be evaluated without special equipment to detect these behavioral changes to an experimental condition. There is a need to develop techniques that have adequate sensitivity to detect changes in oral function but do not require a surgical manipulation or require tethering of the animal to the recording equipment.

The purpose of this study was to evaluate a new technique that is non-invasive, does not require training, can be used in a home cage environment to minimize stress, and assesses incising force in three dimensions over an extended period such as 24 h. Repetitive acidic saline injections were used to create persistent masticatory muscle hyperalgesia and alter incising activity to test the sensitivity of this technique over a 35 day period in male and female mice.

2. Materials and methods

2.1. Animals

Equal numbers of male and female CD-1 mice (total n = 48) from the same distribution source (Charles Rivers) were housed in one animal room with four mice of the same sex housed in the same home cage. Each mouse was individually assessed in an identical home cage located in a sound attenuation chamber (ENV-022V, Med Associates, Inc.) in the same animal room, so minimal relocation of animals was required for the behavioral assessment. All mice were housed and assessed with ad libitum access to water and food and were maintained in a normal 12 h light/dark cycle at an average temperature of 25 °C. Each sound attenuation chamber was ventilated and equipped with the same 12 h light/dark cycle schedule to maintain continuity with the animal room. The animal care and experimental procedures were in compliance and approved by the University of Florida Institutional Animal Care and Use Committee.

2.2. Data acquisition

Audio, video and incising force recordings were acquired during a 24 h period from four cages simultaneously. Video recordings (B&W) were made for the entire 24 h period using a small analog camera (HRC-20P with 6 mm lens, SCS, Enterprises, Inc.) connected to a DVR to assess on-going behavior such as general activity, posture and grooming activities and to validate incising behavior. Three dimensional incising forces were assessed using a multi-axis force transducer (NANO17-E, ATI Industrial Automation) attached to a custom food holder with three pieces of standard chow (Harlan Laboratories). The force transducer-mounted chow was positioned in the standard chow tray and the mouse accessed the chow through the wire top (Fig. 1). Episodes of incising forces/sound were recorded using custom-written software (LabView 2013, National Instruments, Inc.). Analog forces were recorded using an A/D converter (PCIe-6343 X Series Multifunction DAQ, National Instruments, Inc.) at 500 Hz and 16 bit resolution while sound was recorded at 51.2 kHz/channel and with a 24 bit resolution (Model 9234, National Instruments, Inc.) using an industrial microphone (Model 378B02, 1/2″ microphone, bandwidth 3.15 Hz–20 kHz, PCB Piezotronics, Inc.). Force/sound recordings were initiated when the 3-D force resultant (instantaneously calculated from the X, Y and Z forces) exceeded a set threshold (2.4 N) and ended after a pre-determined period of no incising (4 s). Post-processing of the acquired signals was accomplished using custom-written software (LabView 2013, National Instruments, Inc.) to remove baseline bias and digitally filter (bandwidth: 0–31.25 Hz) the individual force axis recordings (X, Y and Z forces), calculate the resultant and automatically identify peak force amplitude and peak time during each episode of incising. Incising episodes with five or fewer incising force peaks were removed from the analysis. Each incising episode was analyzed to determine the instantaneous incising frequency between two force peaks. For each of these pair of peaks, the second peak was evaluated for peak force, and top 10% incising forces. Incising episode duration, the total number of incising episodes, the total number of incisions, weight of chow consumed and body weight were also assessed for each 24 hour recording.

2.3. Pain model description

A persistent mild to moderate jaw closing muscle hyperalgesia was induced by two 20 μl injections of sterile acidic saline (pH = 4.0) into the left masseter muscle separated by five days. Repetitive injections within the 3–7 day window have been shown to elicit hyperalgesia in muscle such as the lateral gastrocnemius that lasts for over four weeks [9]. A control group consisted of two 20 μl injections of sterile neutral saline (pH = 7.0) into the left masseter muscle separated by five days. The injection was made into the center of the muscle as assessed from the anterior/posterior and dorsal/ventral borders of the masseter and the needle was inserted until lightly touching the body of the mandible and then retracted approximately 0.5 mm. Mice were anesthetized using an intraperitoneal injection of xylazine (15 mg/kg) and ketamine (75 mg/kg) prior to the acid/neutral saline injections.

2.4. Incising recordings

Four baseline recordings and seven post-injection recordings (1 day after the first injection and 3, 7, 14, 21, 28 and 35 days after the second injection) were made to assess incising behavior. Mouse weight was monitored at each recording to determine any adverse effects on the mice. During this study, no mouse was ever found to be in overt distress such as weight loss >10%, ruffled fur, persistent face grooming on the side of injection or lack of activity. The animals were weighed, transferred to the home cage for recording and placed in individual sound attenuation chambers by the same male experimenter (CGW).

2.5. Statistical analyses

Incising variables were analyzed for differences between sex and age. When data satisfied the requirement for parametric analyses, a mixed model Analysis of Variance was calculated and, when significant, post-hoc testing was conducted using Fisher's Least Significant Difference (LSD) test. Data not satisfying the requirements of parametric analyses were tested using appropriate non-parametric analyses. A probability level of less than 0.05 was used for all statistical testing. Three age groups of male and female mice were evaluated to determine the effect of age on incising: 3 months (n = 12 per sex for the baseline/hyperalgesia condition; 4 per sex for the control condition), 6 months (n = 4 per sex for