Physiology & Behavior 167 (2016) 132–144

Contents lists available at ScienceDirect

Physiology & Behavior

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

Mouse preferential incising force orientation changes during jaw closing muscle hyperalgesia and is sex dependent

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HIGHLIGHTS

- A multiaxis force transducer was used to assess incising direction preference in a mouse model.
- Sex differences were identified for preferred incising direction during baseline (non-pain) recordings.
- Incising direction preference differed among the discrete incising frequencies.
- Female incising direction preference was more affected than males in a pre-clinical model of pain.

ARTICLE INFO

Article history:
Received 17 June 2016
Received in revised form 22 August 2016
Accepted 8 September 2016
Available online 9 September 2016

Keywords:
Central pattern generator
Mastication
Incising forces
Pain

ABSTRACT

Introduction: Mouse incising is controlled by a central pattern generator and this activity can change in the presence of pain. The incising frequency and maximum force generation decreases with pain. In this study, we used repetitive acidic injections in the left masseter muscle of male and female mice to determine differences between baseline and jaw muscle pain conditions and the effect of sex on preferential incising direction.
Methods: A within subject design was used to evaluate data previously acquired using multi-axis force data (X, Y and Z) from the 4th baseline recording day and day 7 post-injection (day of maximal pain response) for each mouse of each sex. A total of 34 female and male (age 3–9 months) CD-1 mice were evaluated. After mathematically rotating the X and Y axes to align the Y axis to be parallel to the wire struts of the cage top, data were analyzed to determine incising direction preference during baseline (non-pain) and pain (day 7) conditions and between sex. Radar plots of X-Y, X-Z and Y-Z axes depicted the average direction of incising preference between baseline and pain conditions for each sex. Statistical differences among groups were tested using a mixed model ANOVA.
Results: Similar to previous findings, female mice had a more robust difference in incising direction preference when comparing male and female pain conditions and this was most evident in the X-Z axes. The incising frequencies most commonly affected were 5.3, 6.2 and 7.6 Hz. Male mice varied little in their incising direction preference between the baseline and pain conditions. In addition, statistical comparison of ratios of the percent of time spent incising in the Z versus X axes for each incising frequency found that the incising preference was not different when comparing 5.3 and 7.6 Hz frequencies. Finally, female mice used a novel approach to minimize pain while incising by rotating their head and body nearly 180 degrees while males did not use this strategy as frequently.
Conclusions: The preferred incising direction changes in a jaw muscle pain condition and this information can be used to further characterize functional pain in the masticatory muscle system. The changes were dependent on the incising frequency generated by the central pattern generator for incising.

1. Introduction

Masticatory muscle force production during mastication and incising is the result of the cumulative effect of descending cortical inputs onto neurons comprising the central pattern generator (CPG) for mastication as well as integration of sensory inputs from jaw closing muscles, periodontium, temporomandibular joints and intraoral mucosa [1].

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http://dx.doi.org/10.1016/j.physbeh.2016.09.005
0031-9384/

Sensory feedback refines masticatory muscle activity to generate sufficient magnitude and direction of forces to accomplish the task of food breakdown prior to deglutition. The breakdown of different types and hardness of foods is important to maintain the necessary food intake for survival. Assessing relative contributions from non-noxious or noxious sensory inputs on masticatory motor control has been a topic of research for many years [2–10]. However, little is known regarding the effect of noxious inputs such as muscle or joint pain on freely motivated behavior because the effects of pain on masticatory behavior have been difficult to evaluate in a non-invasive approach and over an extended period of time. Pain during mastication or incising can affect the ability to efficiently allow adequate food intake by decreasing maximum masticatory force magnitude and decreasing cycle frequency through the central pattern generator for mastication located in the brainstem [11]. Both animal and human studies have focused on the effects of acute pain and these studies may be limited in the interpretation regarding the effects of persistent pain that lasts for weeks to months to years. Animal studies of experimental jaw muscle pain and temporomandibular joint (TMJ) pain have been conducted using algesic substances injected into the muscle and have been commonly evaluated using reflex studies such as mechanical stimulation or thermal stimulation [12–15]. However, some of these algesic substances, such as Complete Freund's Adjuvant (CFA), cause a prolonged pain and extensive muscle necrosis that does not reflect the typical muscle pain presentation of human subjects. The use of these substances more closely approximates an acute inflammatory pain with deafferentation of peripheral nerves innervating the muscle. Human studies have evaluated the effects of short-term jaw closing muscle pain on masticatory behavior by injection of such algesic substances as hypertonic saline, glutamate or NGF to create masticatory muscle pain [16–18]. The results have been varied possibly due to small sample sizes, presence of a needle or catheter in the jaw closing muscle during active chewing or the high variation observed among the participants [19–23].

Recently, we described a technique to non-invasively record incising forces without experimenter intervention and for long periods of time (24 h) in the home cage environment [10]. In an experimental jaw closing muscle pain condition, female mice were found to respond more robustly to repetitive acidic saline injections into the masseter muscle than male mice with a longer duration of the response and a shift to lower incising frequencies. Assessment of the preferred direction of rhythmic force production during incising during non-noxious and noxious afferent inputs can provide novel information on the effects of peripheral afferent activation on the central pattern generator. In addition, sex differences in response can be assessed during these varied inputs. In this study, two hypotheses were tested. The first hypothesis, based on our previous findings, was that jaw muscle pain would cause a deviation from the normal, non-noxious incising direction preference and that the magnitude of deviation would be sex dependent. A second hypothesis was also based on our previous findings that, of the five incising frequencies identified, there was a high, negative correlation (CC = −0.85) found specifically for the 5.3 and 7.6 Hz incising frequencies. One explanation for this high correlation was the possibility of the bistability of neurons in the incising CPG network. Bistable neuronal activation should elicit a similar mechanical action but at different incising frequencies. Thus, our second hypothesis to be tested was that incising frequency pairs, such as the 5.3 and 7.6 Hz frequencies, would have similar preferred incising directions but would be statistically different from other incising frequencies. Male and female mice were evaluated using a within comparison design during baseline (no pain), repetitive acidic saline injections (pain) and repetitive normal saline control mice (minimal pain) to determine the influence of jaw muscle pain on masticatory muscle motor control during incising.

2. Materials and methods

2.1. Animals

Animals that were used in this study were the same ones as described in a previous report [10]. A total of 34 CD-1 mice (Charles Rivers) consisting of 17 males and 17 females (age 3–9 months) were housed in the same animal room with four male or female mice residing in the same home cage. Mice were individually assessed in a sound attenuation chamber (ENV-022V, Med Associates, Inc.) in the same room as they were housed. All mice had ad libitum access to water and food and were exposed to a 12 h light/dark cycle with the room maintained at an average temperature of 25 °C. Mice were maintained in the sound attenuation chamber with the same 12 h light/dark cycle schedule as the animal room. The University of Florida Institutional Animal Care and Use Committee approved the animal protocol for this study.

2.2. Data acquisition

The data acquisition procedures for this study have already been described in a previous publication [10]. Briefly, audio, video and incising force recordings were simultaneously acquired from four cages during

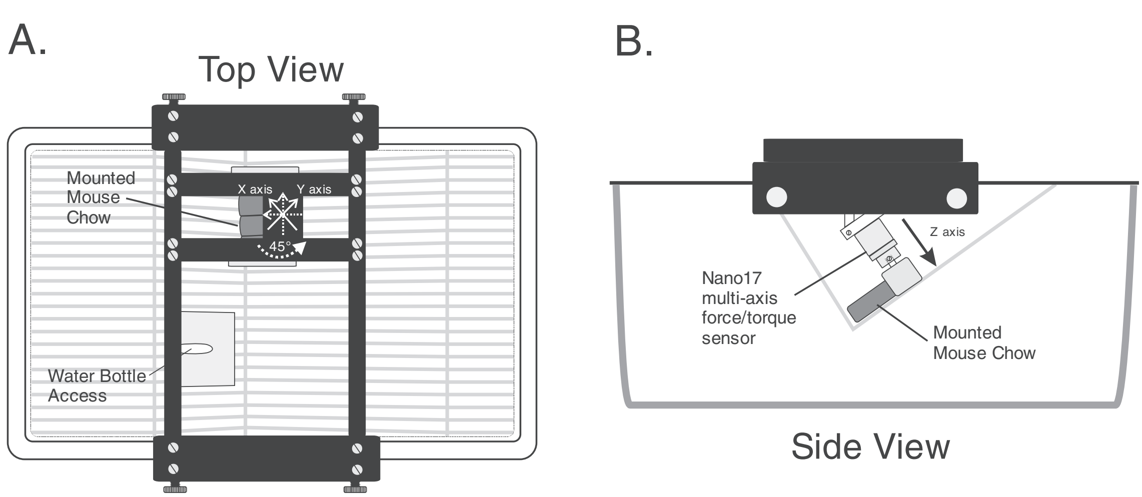

Fig. 1. Position and orientation of multi-axis force transducer mounted on the home cage with mouse chow attached to the transducer. Top view (A) and side view (B) show the orientation of the X, Y and Z axes. White arrows represent X and Y axes and black arrow represents the Z axis. Positive is represented by the direction of the arrowhead. The X axis represents the anterior-posterior direction parallel to the wire slats of the cage top (as the animal faces the food tray normally); the y axis represented the left–right direction; and the z axis represented the opening-closing jaw movement.