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A novel operant-based behavioral assay of mechanical allodynia in the orofacial region of rats

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HIGHLIGHTS

- A novel operant device was used to measure orofacial mechanical allodynia in rats.
- Our device was validated using a capsaicin model of orofacial neuropathy.
- Individual animal behavioral measurements were assessed.
- Morphine attenuation was successfully detected by our device.

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ABSTRACT

Background: Detecting behaviors related to orofacial pain in rodent models often relies on subjective investigator grades or methods that place the animal in a stressful environment. In this study, an operant-based behavioral assay is presented for the assessment of orofacial tactile sensitivity in the rat.
New methods: In the testing chamber, rats are provided access to a sweetened condensed milk bottle; however, a 360° array of stainless steel wire loops impedes access. To receive the reward, an animal must engage the wires across the orofacial region. Contact with the bottle triggers a motor, requiring the animal to accept increasing pressure on the face during the test. To evaluate this approach, tolerated bottle distance was measured for 10 hairless Sprague Dawley rats at baseline and 30 min after application of capsaicin cream (0.1%) to the face. The experiment was repeated to evaluate the ability of morphine to reverse this effect.
Results: The application of capsaicin cream reduced tolerated bottle distance measures relative to baseline ($p < 0.05$). As long as morphine did not cause reduced participation due to sedation, subcutaneous morphine dosing reduced the effects of capsaicin ($p < 0.001$).
Comparison with existing method: For behavioral tests, experimenters often make subjective decisions of an animal's response. Operant methods can reduce these effects by measuring an animal's selection in a reward-conflict decision. Herein, a method to measure orofacial sensitivity is presented using an operant system.
Conclusions: This operant device allows for consistent measurement of heightened tactile sensitivity in the orofacial regions of the rat.

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1. Introduction

Orofacial pain can be caused by multiple disorders, including myofascial pain, headaches, temporomandibular joint disorders, and trigeminal neuralgia; combined, these conditions affect more than 20% of Americans (Lipton et al., 1993). Many orofacial pain conditions have overlapping symptomatic presentations, with symptoms including heightened sensitivity to temperature and touch in the orofacial region. This orofacial hypersensitivity to thermal and mechanical stimuli can be replicated in rodent orofacial pain models (Krzyzanowska and Avendaño, 2012; Kupers, 2001). Unfortunately, rodent orofacial pain models that are used to test emerging pain therapeutics are commonly evaluated with behavioral methods that have limited sensitivity and can be prone to experimenter bias. Accurate quantification of pain-related behaviors in rodent orofacial pain models is crucial for the preclinical evaluation of potential therapeutics and analgesics (Krzyzanowska and Avendaño, 2012).

Animal behavioral analyses can be used to assess the physical and symptomatic consequences of disease in the rodent. Behavioral assessments of orofacial pain in animals commonly include measuring pain-related response to heat (thermal hyperalgesia) or withdrawal response from the application of von Frey filaments (tactile allodynia). In addition, exploratory field behavior, freezing-like behavior, resting/sleeping time, grooming behavior, thigmotactic scanning, and dietary tracking may also be related to behavioral consequences of orofacial pain in animal models (Castonguay et al., 1986; Deseure and Adriaensen, 2002; Kerins et al., 2003; Kramer et al., 2012; Vos and Strassman, 1994). However, determining the sensitivity of the orofacial regions of a rodent can be difficult using some common methods. As an example, fixed stimulus and investigator driven devices can be used to determine tolerance thresholds in the orofacial region based on animal reaction to stimuli (Imamura et al., 1997; Krzyzanowska et al., 2011; Morris et al., 1982; Rosenfeld et al., 1978; Vos and Strassman, 1994); however, variations in reaction scoring criteria between experimenters, animal stress due to handling and restraint, and differences in protocols can limit the sensitivity of these methods and confound the results (Chesler et al., 2002; Hogan et al., 2004; Lambert et al., 2009).

Operant behavioral tests can combine the unobtrusive nature of observational tests with quantifiable thresholds to various stimuli (Mauderli et al., 2000). Existing operant tests for orofacial nociception include dietary and meal duration changes, as well as a device called the dolognawmeter that quantifies length of time to chew through plastic dowels (Dolan et al., 2010; Kerins et al., 2003; Kramer et al., 2012). These devices can describe pain-related behaviors in a variety of induced orofacial nociception models, but do not provide a withdrawal threshold associated with surface nociception similar to von Frey filament protocols. A commonly used operant test design uses a reward-conflict paradigm, in which the animal must accept a stimulus to receive a reward. Through the measurement of an animal's participation, a threshold to the applied stimulus can be determined. Similar operant tests have previously been validated to describe thermal sensitivity (Neubert et al., 2005), and first-generation operant tests of tactile sensitivity have also been prototyped (Nolan et al., 2011). The primary output of these operant tests of orofacial sensitivity is bottle contact time, which is shown to be effective in differentiating naïve and experimental animals. In addition, facial contact with the stimulus and lick/pain indices can be constructed from these tests. However, this design is unable to quantify individual animal withdrawal thresholds. Additionally, in early prototype design, the animal could unequally apply the mechanical stimulus to the orofacial regions by tilting its head.

In this paper, an operant-based test is described for the detection of mechanical sensitivity in the orofacial region of rats. This device allows animals to participate independent of the experimenter, detecting rodent pain-related behaviors through a reward-conflict paradigm (Neubert et al., 2005). Our approach improves prior operant measures of tactile sensitivity in the orofacial region through two keys aspects: First, the stimulus required to receive the reward is increased during a trial, requiring the animal to tolerate increased mechanical force on the face to continue to receive the reward. Second, animal avoidance measures are minimized by incorporating a 360° array of stimulus that cannot be avoided by an animal making contact with the reward bottle. Increasing stimulus and minimizing avoidance errors allows for effective measurement of behavioral changes associated with surface nociception. To validate this approach, pain-related behaviors are quantified through individual animal's tolerance for the mechanical stimulus under conditions of pain and analgesia. This approach could be used to detect changes due to induced pain or the efficacy of an analgesic and can provide a highly sensitive behavior method to screen the efficacy of emerging orofacial drugs and therapeutics.

2. Materials and methods

2.1. Operant-based test of mechanical orofacial sensitivity

The testing procedures and general handling of animals described herein were approved by the Institutional Animal Care & Use Committee at the University of Florida and are in compliance with the ethical guidelines and standards established by the Guide for Care and Use of Laboratory Animals (National Research Council, 2011).

2.1.1. Device and function

An orofacial sensitivity device was designed to function on a reward-conflict paradigm, similar to that of previous operant devices (Mauderli et al., 2000; Neubert et al., 2005). The device consisted of a 7.5″ × 7.75″ × 5.75″ acrylic testing chamber with a 2.185″ × 2″ window and removable metal floor. A 360° array of looped 0.010″ diameter stainless steel wire with a 0.7″ opening at the center of the array was used to partially block the window (Fig. 1**a**). A reward bottle filled with diluted sweetened condensed milk (2 Water: 1 Sweetened Condensed Milk, Nestlé, La Lechera) was placed inside the window, such that the 360° array of wires would impart a mechanical stimulus to the orofacial regions of a rat drinking from the reward bottle (Fig. 1**b**). Initially, the reward bottle nozzle sits inside the array, such that initial contact with the bottle does not require contact with the mechanical stimulus. Contact with the reward bottle completes an electric circuit between the cage floor, animal, and bottle, allowing for the measurement of contact time (100 Hz, custom LabVIEW 2013 module, National Instruments). In addition, continuous contact with the reward bottle, defined as 15 out of 30 ms of contact time, initiates a stepper motor underneath the cage floor to move the bottle further from its initial position at a rate of 5.0″/min (Fig. 1**c**). This movement of the water bottle slowly increases the distance between the reward bottle and cage, requiring the animal to tolerate more mechanical force to continue to receive the reward. The bottle circuit remains dynamic for a period of 2 min, after which point the tolerated bottle distance is recorded and the bottle is automatically returned to its starting position. If the bottle position has changed from its initial position during a 2 min time period, the period is considered a drinking event and indicates participation.

2.1.2. Testing protocol

Animals were food fasted for 12–15 h (overnight) prior to testing. The following morning, animals were placed in the acrylic