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Short communication

# Adaptation of a novel operant orofacial testing system to characterize both mechanical and thermal pain

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ABSTRACT

Mechanical pain sensitivity is characteristic of many orofacial pain conditions; however, few models exist to quantify this pain. Here we evaluated a novel adaptation of our existing operant system to characterize orofacial pain following mechanical and thermal stimuli. We demonstrate that the operant system is able to detect painful and analgesic responses to mechanical stimuli. These findings allow comparison of both mechanical and thermal stimuli using the same outcome measures.



Clinically many orofacial pain complaints including trigeminal neuralgia, tooth extractions, and pain associated with orthodontic tooth movement involve a mechanical component (i.e., touch sensitivity or pain with injury). Although the characteristics of clinical orofacial pain are well described, evaluation of orofacial pain in animals is challenging. Previously, assessments of trigeminal nerve-mediated nociceptive responses have been limited to methods that assess processing within the brain stem [5,10,17,23], utilizing unlearned behaviors that were elicited by mechanical sensitivity using von Frey filaments [23] or thermal stimulation [9]. Our lab has previously reported the development of a novel thermal operant facial testing system that provides an investigator-independent behavioral assessment system (Fig. 1A). We use operant conflict paradigms to establish a behavioral outcome in which an animal can decide between receiving a reward and escaping an aversive stimulus [13,18]. The current report utilizes a modification of our original operant test system by adding a simple mechanical component. This new modification provides investigators with an opportunity to assess both mechanical and thermal assessments of facial allodynia, hyperalgesia, and pain using the same operant system that we developed previously [15]. This novel mechanical behavioral assessment strategy of orofacial pain could further provide a key step for the advancement of translational pain research, as we now demonstrate the ability to directly assess and compare mechanical versus thermal pain using the same outcomes.

The objective of this study was to characterize behavioral responses to facial mechanical stimulation and to see how these responses change under conditions of inflammation and analgesia. Since we are using an adaptation of our established thermal facial operant testing paradigm, we also compared baseline thermal stimuli data (unpublished) collected over the past 6 months using our standard thermal operant system, as described in detail previously [15]. In this current study, we modified standard nickel titanium (NiTi) wires similar to those used in orthodontics for providing tooth movement. The advantage of nickel titanium wires is they have tremendous memory and strength, thus allowing for standardized repeated use of the same wires. Additionally, these wires can conduct electricity, so we were able to modify our existing cage to detect and record actual facial contacts, which is one of the keys to the operant testing. We examined the influence of two diameters of nickel titanium wires (0.010 in. and 0.007 in.) on

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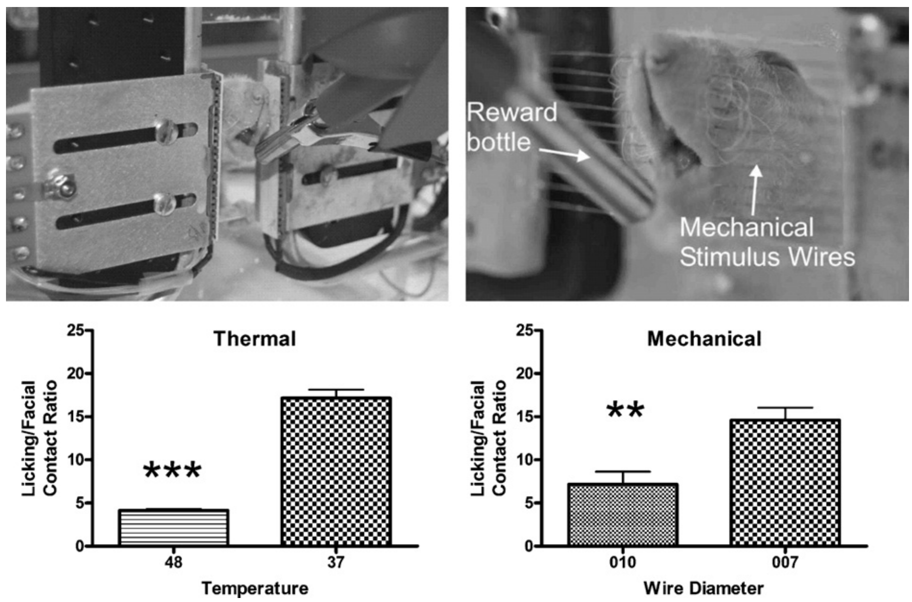


**Fig. 1.** Comparison of a thermal and a mechanical operant testing system. Photograph of a rat performing a thermal (A) and mechanical (B) facial operant test. In each test, the rat's cheeks must contact the thermode or stimulus wires in order to reach the reward bottle containing sweetened condensed milk. Comparison of the Lick/Face contact pain ratio for thermal (C) and mechanical (D) stimuli demonstrated that there was a significant decrease in the Lick/Face ratio when the stimulus thermode was set at 48 °C ($N = 134$) as compared to 37 °C ($N = 82$; $^{***}p < 0.0001$). There was a similar decrease in this ratio when comparing NiTi wires with a diameter of 0.010 in. ($N = 10$) and 0.007 in. ($N = 10$; $^{***}p < 0.005$).

the performance of a facial reward/conflict paradigm testing system [15,16,19]. We have previously published and presented some of this data in abstract form [8] and this study builds on this prior work by directly comparing the effects of thermal versus mechanical stimuli on the same operant outcome measure.

Adaptation of the operant orofacial system was accomplished by adding two horizontal rows of NiTi wires embedded in an acrylic module that could be easily adjusted, attached and removed from our existing operant testing boxes. Unrestrained hairless male Sprague–Dawley rats (250–300 g, Charles River, Raleigh, NC) were housed in a standard environment. During testing, animals were placed separately in each operant cage and the reward bottle containing diluted (1:2 with water) sweetened condensed milk solution (Nestle, room temperature) was positioned in proximity to the cage such that the animal will be allowed access to the reward bottle when simultaneously contacting the NiTi wires (either 0.010 in. or 0.007 in. in diameter, (Small Parts, Lexington, KY)) with its face (Fig. 1B). Both the metal spout on the watering bottle and the NiTi wires of the faceplate was connected to a DC power supply and, in series, to a multi-channel data acquisition module (WinDaq Data Acq DI-710-UH, DATAQ Instruments, Inc.). As with our prior thermal test, when the animal completed the task and drank from the bottle, the animal's tongue contacted the metal spout on the water bottle, completing an electrical circuit. The closed circuit was registered in the computer and each spout contact was recorded as a licking event. A separate circuit was established from the NiTi mechanical wires to the animal by grounding the floor with an aluminum sheet for recording of facial contact events. We previously tried unsuccessfully to use proximity sensors to detect actual facial contacts, as animals are able to modify their strategy to accessing the reward and thus leading to potential false positive responses. Therefore this latter facial contact circuit is critical and necessary to determine if the animal is discouraged by the stimulus. The duration of each facial contact and the total number of events (licking, facial contact) were recorded. During offline data analysis the cumulative duration and frequency of events were determined for both the licking (reward) contact data and the facial stimulus data. A licking/facial contact ratio was calculated for each animal and presented as mean ± s.e.m., as described previously [15]. When considering this ratio in the context of behavioral pain testing, a non-nociceptive stimulus (e.g., 37 °C) typically results in a high number of licking events per single facial contact for the animal. Under nociceptive conditions (e.g., 48 °C), this ratio significantly decreases, as the animal is only able to achieve a low number of licking events per single facial contact. Alternatively, the animal must significantly increase the number of facial stimulus contacts to achieve the same number of reward licking events. We compared the effects of each wire (0.007 in. vs. 0.010 in.) and neutral and hot thermal stimuli on these outcome measures.

We also examined the effect of neurogenic inflammation and opioid administration on the performance of the operant test for each wire diameter. A capsaicin cream (0.075%, Thomson Micromedix, CO) was liberally applied to the cheek region of lightly anesthetized (inhaled isoflurane, 1–2%) rats and left on for 5 min to produce nociceptive sensitization [14]. The capsaicin was then removed and the face was wiped clean with a moist paper towel and returned to their home cage for 30 min prior to behavioral testing. The final experiment studied the effect of morphine analgesia on performance in the operant test. Morphine (Baxter Healthcare Corporation, Deerfield, IL) was diluted in phosphate buffered saline pH 7.4 (PBS) and injected subcutaneously (1 mg/kg, volume of 200–250 μl) 30 min prior to testing. All rats had *ad libitum* access to food and water between testing sessions and their weights were monitored weekly but were food fasted for 16 h prior to each testing session and trained ($n = 9$ sessions) to drink sweetened condensed milk. This lead-in training period was necessary to acquaint the animals with the task of locating the reward bottle.

Statistical analysis included an unpaired $t$-test to compare thermal (37 °C vs. 48 °C) and a paired $t$-test to compare mechanical (0.007 in. vs. 0.010 in.) baseline data. Additionally, comparison of the 0.007 in. and the 0.010 in. data was performed using a one-way ANOVA with a Dunnett's post-test with the baseline data of each group used as the control for comparison. All statistical evaluations were made using GraphPad Prism (v. 4.02, GraphPad Software, San Diego, CA). Significance was set at $p < 0.05$.