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Behavioural Brain Research 170 (2006) 308–315
BEHAVIOURAL BRAIN RESEARCH
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Research report

Differentiation between capsaicin-induced allodynia and hyperalgesia using a thermal operant assay

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Received 23 November 2005; received in revised form 28 February 2006; accepted 7 March 2006
Available online 3 May 2006

Abstract

Investigations of new analgesic treatments ideally are coupled with the use of compassionate methods for pain testing in animals. Recently, we described a novel operant thermal testing device that can be used to quantify orofacial pain. The objective of the current study was to differentiate thermal allodynia from hyperalgesia using this operant thermal assay.

Rats were trained to complete a task whereby they had a conflict between a positive reward and tolerance for thermal nociceptive stimulation. They were subjected to cool to hot temperatures (24–45 °C) and evaluated under naïve (untreated), capsaicin cream (0.075%), capsaicin/morphine, or morphine test conditions. The following outcome measures were evaluated: reward intake; licking contacts; facial contacts; time to complete 25, 50, and 75% of the events (licks and face contacts); facial contact duration; ratio of reward/stimulus contacts; and ratio of facial contact duration/event.

Capsaicin produced an increase in mechanical sensitivity and a significant thermal allodynic effect at 42 °C and hyperalgesic effect at 45 °C. These effects were blocked with morphine pre-treatment. The temporal profile for completing the task was also significantly altered following capsaicin treatment. These data demonstrate that using the operant orofacial assay in conjunction with capsaicin cream can provide a reproducible, sensitive, minimally invasive, and powerful approach for quantifying and studying enhanced thermal pain within the trigeminal system. This technique provides an alternative to reflex tests of orofacial sensitivity, and it presents a pivotal link for translating basic pain research into clinic trial strategies.

Keywords: Pain; Operant behavior; Orofacial; Thermal sensitivity; Capsaicin

1. Introduction

Assessment of orofacial pain in animals has been limited to a handful of methods that rely upon segmental (e.g., withdrawal responses) or brainstem (e.g., grooming) processing [3,7,16,23]. This work provided a foundation for studying orofacial sensitivity in animals; however, these approaches supply limited information regarding trigeminal pain modulation and higher order processing. We recently validated and reported the use of a novel operant thermal testing system for assessing orofacial pain [13]. Using a reward-aversion paradigm, we demonstrated that this assay discriminates between painful stimulus intensities, as shown by thermal-stimulus response functions.

The use of algesic agents to induce nociceptive sensitivity represents a necessary step in evaluation of new behavioral testing paradigms [22]. Capsaicin, one of the primary ingredients of red hot chili peppers [6], specifically excites nociceptive C-fibers of sensory neurons and has been widely used as an algogenic agent in pain studies [5,11,14,20]. For example, injection of capsaicin has been shown to produce a primary heat and mechanical hyperalgesia with subsequent development of secondary hyper-

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doi:10.1016/j.bbr.2006.03.008

algesia and allodynia in humans [11,18,19]. Additionally, topical capsaicin has previously been used as a model for producing a repeatable, non-distressing, transient thermal and mechanical hyperalgesia, as determined by operant testing in monkeys [10]. Nociceptive reflexes are also enhanced in monkeys following capsaicin application [2], and opioid receptor agonists have been shown to attenuate capsaicin-induced enhancement of reflex responses to thermal stimulation [1,8,9]. The effect of morphine on pain sensitivity after capsaicin application is largely due to reduction of central effects of unmyelinated C-fiber nociceptors input [24]. However, morphine dosage is an important consideration. Vierck et al. [21] have demonstrated a dichotomous effect of morphine on pain behaviors, with low doses attenuating operant outcomes while increasing reflex responses.

Distinguishing between thermal allodynia and hyperalgesia requires the ability to demonstrate enhancement of behavioral responses to both non-noxious and noxious stimulus temperatures. Recently, we demonstrated using an operant orofacial testing paradigm that there is a differential behavioral response at non-noxious ($\leq 41\,^{\circ}C$) versus noxious temperatures ($\geq 45\,^{\circ}C$). As proof of principle, we used carrageenan to induce a gross inflammatory insult that produced a thermal hyperalgesic response at 45 °C and this response was reversed by morphine. While this study was successful in initially characterizing the operant orofacial testing system, there are limitations with using carrageenan for modeling orofacial pain. Animals swell significantly and preclude the expeditious retesting of animals, which may inhibit investigators from completing within-subject or test–retest comparisons of experimental therapies. The aim of the current study was to build on these initial findings by using capsaicin as a non-damaging, mild stimulus in order to distinguish thermal hyperalgesic from allodynic responses. We assert that a low dose capsaicin cream (0.075%), in conjunction with the thermal operant testing device can provide a reproducible and sensitive model for evaluating heat sensitivity. This is important because it provides a means of repeatedly testing animals and comparing their behavioral responses under a variety of conditions, thus allowing for screening of potential analgesic agents.

2. Methods

2.1. General

Male Sprague–Dawley rats (200–300 g, $N = 40$) were lightly anesthetized 1 day prior to testing (isoflurane, 1–2.5%, inhalation), and the face was shaved using clippers, followed by application of depilatory cream, and excess cream was removed with a moistened paper towel to minimize skin irritation. Rats were food fasted (12–15 h) prior to each testing session but were provided with standard food chow immediately following each session and on non-testing days. Animals were brought into the behavioral procedure room 1 h prior to testing at the same time each day and allowed to acclimate to the temperature and ambient noise of the room. In order to minimize nutritional differences from their normal food routine, a recovery day or two was included between each session, and water was made available ad libitum when animals were not in a testing session. Animal weight was recorded daily. Animal testing procedures and general handling complied with the ethical guidelines and standards established by the Institutional Animal Care & Use Committee at the University of Florida, and all procedures complied with the Guide for Care and Use of Laboratory Animals [4].

2.2. Thermal testing

Assessment of thermal sensitivity was completed using an orofacial operant thermal assay, as described previously [13]. Briefly, an acrylic testing cage (20.3 cm W × 20.3 cm D × 16.2 cm H) was constructed that had a 4 cm × 6 cm opening in one wall which was lined with grounded aluminum tubing. This tubing served as a thermode when connected to a water pump (NES Laboratories Inc.) via flexible polyethylene tubing through which cooled or heated water (range: 24.3–45.5 °C) was circulated. A standard rodent watering bottle containing a diluted sweetened condensed milk solution (1:2 with water, room temperature) was mounted outside the cage. The circulating water pump was activated and the appropriate temperature was set prior to testing the animals. The room temperature was maintained at $22 \pm 1\,^{\circ}C$ for all behavioral tests.

Unrestrained animals were placed into separate testing cages, and the data acquisition system was activated (DATAQ Instruments Inc.). The bottle was then positioned such that the animal was allowed access to the reward bottle when simultaneously contacting the thermode with its face. Both the metal spout on the watering bottle and the thermode bars were connected to a dc power supply and, in series, to a multi-channel data acquisition module (DATAQ Instruments Inc.). When the rat drank from the water bottle, the skin on its shaved face contacted the grounded thermode, and 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 data were collected at 60 Hz for the entire length of the experiment. The bottle position was adjusted horizontally and vertically to facilitate contact of the thermode within the same shaved area of the face for each animal. Each spout contact was recorded as a "licking" event, and a separate circuit was established from the metal thermode to the animal by grounding the floor with an aluminum sheet for recording of "facial contact" events. The latter circuit was necessary to determine if the animal made an attempt at the reward bottle but was discouraged by the temperature of the thermode. The duration of each facial contact and the total number of events (licking and facial contact) were recorded. The investigator monitored online data acquisition to ensure that each recorded licking event from the first circuit corresponded to a recorded facial contact on the tubing (the second circuit). This ensured that the animal did not access the reward while avoiding the thermode, and it minimized false-positive recordings of licks. Animals were first trained to drink milk while contacting the thermode that was set to a temperature at 37 °C for baseline training ($N = 5$ sessions). The criterion for rats completing milk-training sessions was established previously [13]: >10 g of reward milk intake to consider an animal as trained, and all animals reached this threshold before further experimental testing. Milk consumption was determined by weighing the milk container using an electronic balance before and after each testing session. Rats were typically tested at 37 °C at the beginning of the week to reintroduce them to the system and then tested under other conditions on an every other day basis for the remainder of the week.

2.3. Orofacial neurogenic inflammation and morphine administration

Capsaicin cream (0.075%, Thomson Micromedix, CO) was used to produce nociceptive sensitization. Capsaicin was liberally applied to all of the shaved areas of the facial region of unanesthetized rats and left on for 5 min. The animals were gently restrained to prevent grooming and subsequent intraoral capsaicin exposure. The capsaicin was then removed and the face was wiped clean with a moist paper towel. Immediately after capsaicin removal, animals were tested at 24.3, 38.0, 42.0, and 45.5 °C, and a random order was used for these testing temperatures. The temperature of the thermode was verified using a contact thermometer (TC-324B Temperature Controller, Warner Instruments Inc.). One set of animals ($N = 7$) was treated in the same manner but also was administered morphine (s.c.: 0.5 mg/kg, 200 μl) between the scapulae 30 min prior to thermal testing. Note that the 0.5 mg/kg dose was chosen to minimize side effects such as sedation. These animals were then tested using the thermal operant device in separate sessions at 42.0 or 45.5 °C.

2.4. Mechanical sensitivity measurement

Assessment of mechanical reflex sensitivity was accomplished using von Frey anesthesiometer monofilaments (Somedic, Sweden) as described previ-

ously [17]. Briefly, rats were allowed to gently rest in one of the examiners hand, and the monofilaments were presented in an ascending series, starting with filament #10 (0.99 g). The filament was applied perpendicular to the skin over the body of the superficial masseter muscle, and a head withdrawal reaction was considered the endpoint response. Each monofilament was applied five times on each side of the face (every few seconds) in an alternating fashion. Successive filaments were applied until the criterion of 60% (three out of five attempts) positive responses were met. A descending series was used if the animal responded at the first filament.

2.5. Data analyses

During offline data analysis, the threshold for detection of facial contacts and licking contacts was set at 1.0 V, above background noise, to minimize false positive event registration, and events typically registered as >5.0 V. An event (licking or facial contact) was registered when the signal went above threshold and ended when the signal dropped below threshold. The total number of events was determined for both licking (reward) contacts and facial stimulus contacts, and a quartile comparison was made evaluating the time to complete 25, 50, and 75% of the events (licks and face contacts) under varying treatments at a testing temperature of 45 °C. The cumulative facial contact duration was also computed.

Two pain indices were calculated by evaluating: (1) the ratio of reward events/facial contact events and (2) the duration per contact with the facial stimulus. Lastly, at the end of each session, the total amount of milk intake (g) was measured, and this value was compared at each of the testing temperatures. Data analyses were achieved using custom-written routines in LabView Express (National Instruments Corporation) and Excel (Microsoft).

2.6. Statistical analyses

Statistical analyses were completed (SPSS Statistical software, SPSS Inc.) to evaluate the outcome measures at the various testing conditions. An ANOVA was used to evaluate the effects of temperature or treatment on each outcome measure, and a general linear model multivariate analysis was used to assess the effects of treatment and temperature. An ANOVA was used for the quartile comparisons of the lick and facial contacts to evaluate treatment effects. When significant differences were found, post hoc comparisons were made using the Tukey HSD or Dunnett's test. The mechanical sensitivity (von Frey) data were not normally distributed; therefore the Kruskal–Wallis test was used to determine overall group differences, and the Mann–Whitney *U*-test was used to evaluate significant differences between test groups. $^{*}P < 0.05$ was considered significant in all instances.

3. Results

3.1. The effects of orofacial neurogenic inflammation on operant outcome measures

Previously we found that the operant outcome measures (intake; reward licking events; facial contact events; facial contact duration) provided a sensitive means for characterizing pain in the facial region [13]. Naïve animals were initially evaluated at each testing temperature (24–45 °C), demonstrating that there was a significant effect ($^{*}P < 0.001$) of temperature for each outcome measure (Fig. 1, white bars): intake [$d.f._{(3,109)}$, $F = 6.830$]; reward licking events [$d.f._{(3,107)}$, $F = 9.258$]; facial contact events [$d.f._{(3,107)}$, $F = 9.179$]; facial contact duration [$d.f._{(3,107)}$, $F = 20.001$]; ratio licking/facial contacts [$d.f._{(3,107)}$, $F = 10.432$]; ratio duration/facial contacts [$d.f._{(3,107)}$, $F = 12.235$]. These results are similar to our previous findings at the same temperatures, with the animals displaying aversive behaviors to the higher temperatures.

In order to induce a neurogenic inflammation, capsaicin cream was liberally applied to the thermal testing zone on the face. Analyzing across temperatures, the animals displayed increased aversive behavior following capsaicin application, as noted by a significant decrease ($^{*}P < 0.001$) in outcome measures (Fig. 1, black bars): intake [$d.f._{(3,73)}$, $F = 13.393$]; reward licking events [$d.f._{(3,73)}$, $F = 10.127$]; facial contact duration [$d.f._{(3,73)}$, $F = 14.368$]; ratio licking/facial contacts [$d.f._{(3,71)}$, $F = 7.980$]; ratio duration/facial contacts [$d.f._{(3,71)}$, $F = 13.923$]. There was a significant interaction ($^{*}P < 0.001$) between testing temperature and capsaicin treatment for: intake [$d.f._{(3,176)}$, $F = 10.377$], reward licking events [$d.f._{(3,174)}$, $F = 8.969$], facial contacts [$d.f._{(3,174)}$, $F = 6.597$], facial contact duration [$d.f._{(3,174)}$, $F = 16.404$], and on the ratio duration/facial contacts [$d.f._{(3,172)}$, $F = 5.319$].

When evaluating the effects of capsaicin compared to naïve (untreated) animals at each individual temperature, we found that a number of the outcome measures significantly decreased at warm (42 °C) and hot (45 °C) thermode temperatures following capsaicin application. This included reward intake, facial contact duration, reward licking events, ratio licking/facial contacts, and ratio duration/facial contacts. At the neutral temperature (38 °C), capsaicin produced a significant decrease in facial contact duration, reward licking events and facial contact events; however, the derived pain indices did not differ between groups (Fig. 1E and F). When the thermode was adjusted to a cool temperature (24 °C), capsaicin produced a significant increase in reward intake, and facial contact duration, compared to untreated animals.

3.2. Effects of morphine on orofacial operant outcome measures

Naïve animals were evaluated at 45 °C as the baseline condition, and morphine was administered 30 min prior to thermal testing, with or without application of capsaicin. Overall, there was a significant treatment effect (naïve, capsaicin, morphine/capsaicin, and morphine) on all six of the outcome measures ($^{*}P < 0.001$, except where noted): intake [$d.f._{(3,94)}$, $F = 15.56$]; reward licking events [$d.f._{(3,97)}$, $F = 19.34$]; facial contact events [$d.f._{(3,97)}$, $F = 14.85$]; facial contact duration [$d.f._{(3,97)}$, $F = 12.25$]; ratio licking/facial contacts [$d.f._{(3,95)}$, $F = 5.39$, $^{*}P < 0.005$]; ratio duration/facial contacts [$d.f._{(3,95)}$, $F = 10.82$]. Morphine significantly blocked the thermal hyperalgesia produced by capsaicin, as indicated by an increase of all outcome measures except facial contact events (Fig. 2). There was a similar trend in the reduction of thermal allodynia by morphine when animals were challenged with capsaicin at 42 °C (Table 1).

There was a significant delay in the time required to reach 25, 50, and 75% of the cumulative events for both licking and facial contacts (Fig. 3) for animals treated with capsaicin. For reward licking events, animals in the morphine/capsaicin condition were able to reach these milestones faster than after capsaicin alone; however, the former group required more time compared to non-capsaicin treated animals. Fig. 3B–D is a representative example of data tracings recorded from an

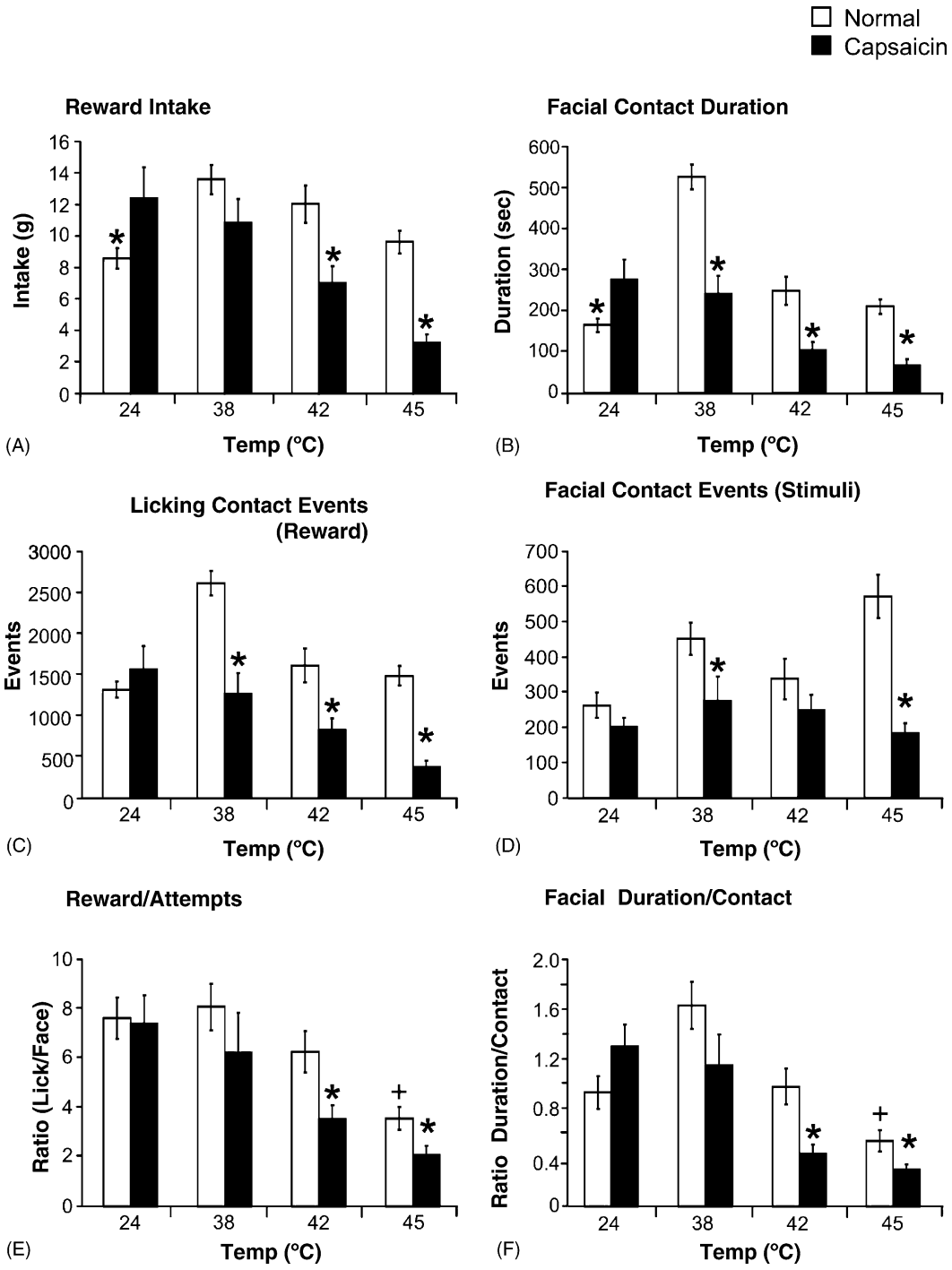

Fig. 1. Effects of temperature and capsaicin treatment on operant outcome measures. We evaluated six outcome measures (A, intake; B, facial contact duration; C, licking contacts; D, facial contacts; E, ratio licks/contact events; and F, ratio facial duration/facial contacts) with and without topical capsaicin treatment at 24, 38, 42, and 45 °C. There was a significant temperature effect (ANOVA, $^{*}P < 0.01$) on all of the outcome measures under non-capsaicin (normal) conditions. The two derived pain indices (E and F) demonstrated a significantly lower ($^{+}P < 0.05$) value as compared to the 24, 38, 42, and 45 °C sessions. Following capsaicin (0.075% cream) application, all outcome measures except facial contact event (D), demonstrated a significant temperature effect. Comparisons between treated and untreated animals at each of the temperatures demonstrated a significant decrease in outcome measure values following capsaicin application ($^{*}P < 0.05$). An exception was noted at 24 °C for intake (A) and facial contact duration (B), as these values were higher than under normal conditions.

individual animal, demonstrating the difference in treatment effects, with the naïve condition presented in (A). Note the multiple facial thermode contacts following capsaicin treatment (B) without corresponding reward contact success. This phenomenon was reversed when the animal was pre-treated with morphine (C). These data demonstrate that a clinically relevant dose of morphine can diminish the development of thermal hyperalgesia produced by neurogenic inflammation.

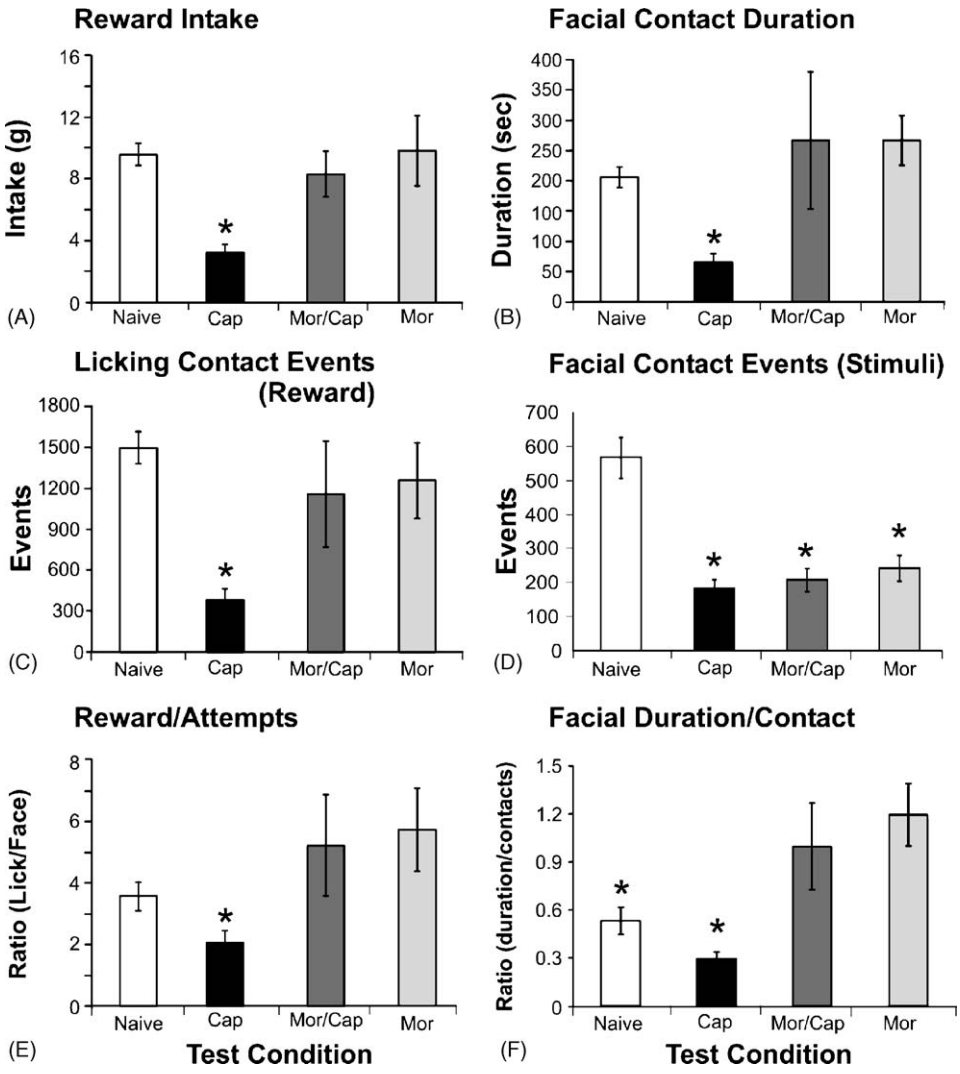

Fig. 2. Effects of morphine on operant outcome measures. The hyperalgesic effect of capsaicin produced at 45 °C was completely blocked when animals were pre-treated with morphine (0.5 mg/kg, s.c.) 30 min prior to testing, as denoted by significantly *higher* values ($^{**}P < 0.05$). Animals treated with morphine, but not capsaicin, behaved similarly in this operant-based assay as compared to the untreated, naïve animals.

3.3. Capsaicin produces a mechanical orofacial allodynia (Fig. 4)

We verified that 0.075% capsaicin enhanced mechanical sensitivity through use of a reflex assay (head withdrawal response to stimulation with von Frey filaments). Following application of topical capsaicin, there was a significant decrease in the head withdrawal threshold. Following capsaicin/morphine administration, there was a significant increase in threshold, compared to capsaicin stimulation. The head withdrawal threshold was also significantly greater for morphine-treated animals, as compared to naïve animals.

Table 1
Effects of morphine on operant outcome measures at 42 °C

Outcome measure	Naïve	Capsaicin	Morphine/ capsaicin
Reward intake	12.02 ± 1.16*	7.08 ± 0.98	6.85 ± 0.94
Licking contact events	1623 ± 215*	865 ± 129	1224 ± 278
Facial contact events	337 ± 61	247 ± 41	263 ± 32
Facial contact duration	244 ± 34*	110 ± 20	301 ± 54*
Reward/attempts	6.24 ± 0.88*	3.63 ± 0.53	4.81 ± 1.70
Facial duration/contact	0.97 ± 0.15*	0.46 ± 0.08	1.29 ± 0.32*

Morphine reduced the thermal allodynic effects produced by capsaicin when animals were tested at a warm temperature (42 °C).
* Significantly higher compared to the capsaicin-treated group, $P < 0.05$.

4. Discussion

The ability to model components of pain including allodynia and hyperalgesia represents a key step in developing new analgesic treatment strategies. While pain in the orofacial region has many of the same components as seen elsewhere in the body, including thermal and mechanical allodynia and hyperalgesia, orofacial evaluation of these components in animals has proved to be challenging. We recently described a new operant method for evaluating thermal sensitivity in the orofacial region that utilized a reward-conflict test paradigm

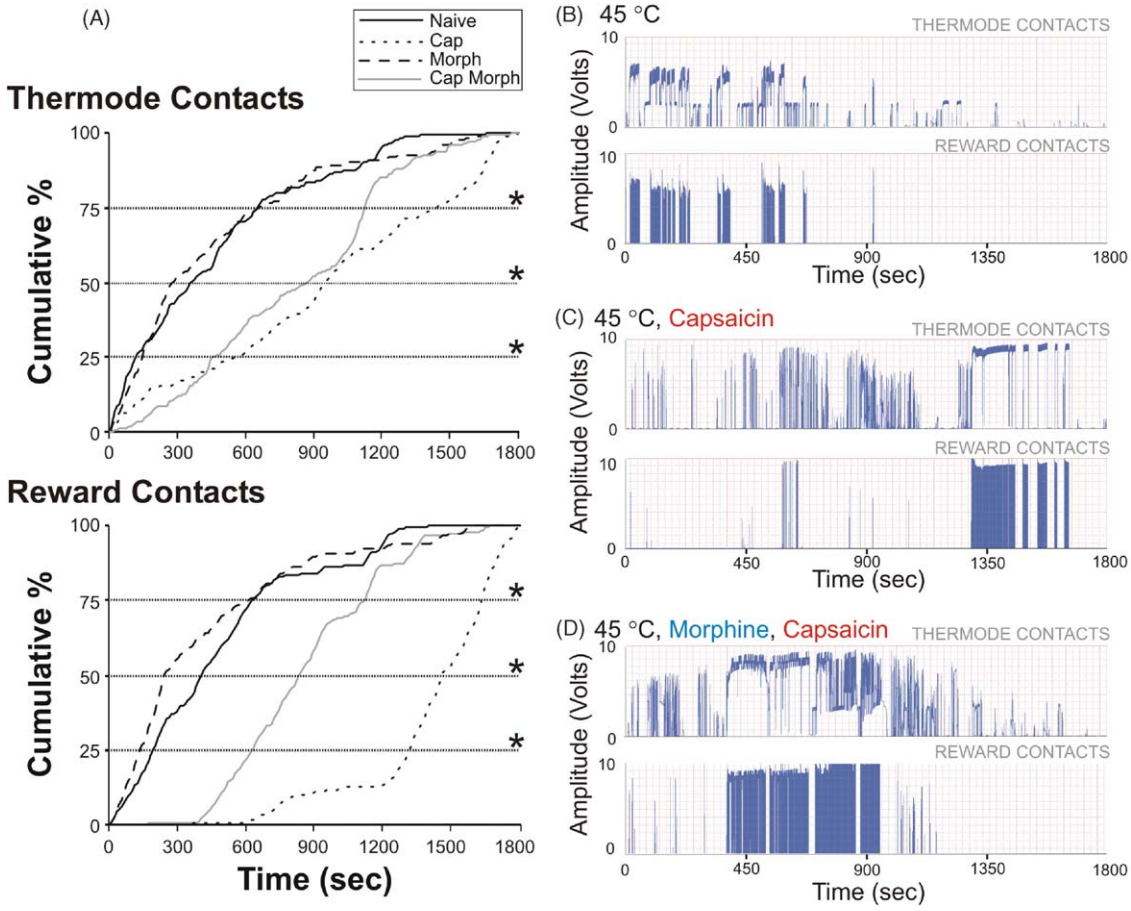

Fig. 3. Temporal operant responses derived at 45.5 °C under varying treatment conditions. There was an overall significant effect of treatment on both thermode contacts (A, upper trace) and reward contacts (A, lower trace) at each quartile ($^{*}P < 0.001$). The thermode contacts were similar for the naïve and morphine-treated animals, but delayed when capsaicin was applied, even in the presence of morphine. When evaluating reward contacts, there was also a delay in the capsaicin-treated groups; however, morphine attenuated this response, with the morphine/capsaicin animals recording successful attempts in approximately half the time as the capsaicin-only group. Examples of trace recordings are from the same animal tested on separate sessions following no treatment (A), capsaicin cream (B), and morphine + capsaicin cream (C). In the upper panel of (B), note the large number of thermode contact attempts following capsaicin treatment and the delay of reward contacts towards the end of the trial. In contrast, for panel (C), the reward contact activity begins much earlier and more prominently, indicating a hypoalgesic response induced by morphine.

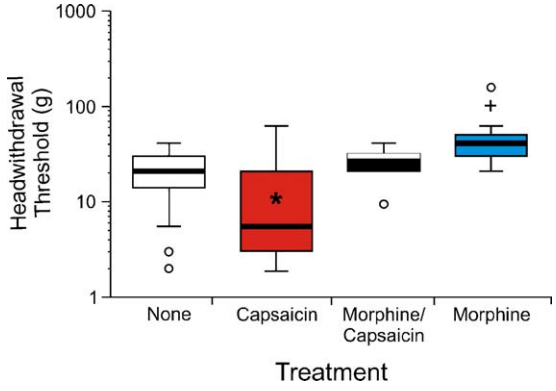

Fig. 4. Reduction of head withdrawal responses are found following bilateral facial topical capsaicin application. Capsaicin (0.075%, topical, 5 min) produced a significant decrease ($^{*}P < 0.001$) in this reflex-based outcome measure, as compared to untreated animals. Morphine administration (0.5 mg/kg, s.c.) blocked development of the capsaicin-induced lowering of thresholds; however, morphine alone also produced a significant increase ($^{+}P < 0.05$) in head withdrawal threshold as compared to normal (untreated) and capsaicin-treated animals. Open circles represent outliers. Note that the head withdrawal response threshold is expressed as the median force (g).

[13]. We found that temperatures ≥45 °C were capable of producing an aversive/painful response using the thermal operant assay, and thermal hyperalgesia developed following inflammation [13]. In the current study, we built on these findings and further characterized the sensitivity of the operant orofacial assay using topical capsaicin as the stimulus.

Capsaicin is known to produce thermal and mechanical allodynia [2,10]; however, it is typically used in amounts greater than 1% when used topically. By varying the testing temperature, we were able to delineate heat hyperalgesia from heat allodynia using a relatively low dose (0.075%) of capsaicin cream. This low dose was chosen to illustrate that stimulation of C nociceptors with a mild chemical stimulant can induce heat hyperalgesia/allodynia. We interpret the decrease of intake, facial contact duration, licking reward contacts, ratio of reward/attempts, and ratio of facial duration/contacts produced by capsaicin as a thermal allodynic response to 42 °C, which does not normally interfere with feeding in the operant test. Also, decreases in these outcomes measures at 45 °C demonstrate development of the thermal hyperalgesia.

When we looked at the raw data traces for naïve, untreated animals or those treated with either capsaicin or capsaicin/morphine, we found that different patterns of accessing and feeding were qualitatively apparent. As seen in Fig. 3C, treatment with capsaicin produced a delay in the successful reward contacts relative to the naïve testing session, even though this animal made multiple attempts on the thermode. Interestingly, there was a shift of the response between the naïve and capsaicin treatments following morphine administration. These qualitative differences prompted us to complete additional analyses to evaluate the temporal shift in responses following each treatment (Fig. 3A).

When evaluating the quartiles for cumulative task completion, there was a significant delay for both thermode and reward contacts following capsaicin relative to naïve or morphine treated animals. Note that it took nearly 600 s for animals to be able to begin to achieve successful licks after capsaicin administration, even though their thermode contacts began within the first few seconds of the start of the session. This indicates that the animals were not simply distracted (i.e., grooming) or incapable of completing the operant task, rather that capsaicin enhanced pain discouraged them from completing the task. When animals were treated with morphine and then challenged with capsaicin, they displayed increased responding relative to capsaicin alone but a delay in their cumulative responses for thermode and reward contacts relative to morphine alone or baseline. This indicated that the low dose of morphine did not completely block the painful response to capsaicin but rather blunted it.

There are several possibilities for an increase in outcome measures at noxious temperatures following morphine administration: (1) altered feeding effects (e.g., increased appetite); and (2) hypoalgesia. Alteration of feeding behavior is unlikely a factor at the low dose of morphine administered, given the fact that the total reward intake did not change following morphine administration. Additionally, as seen in Fig. 3A, the cumulative traces for the naïve and morphine groups are nearly identical. Thus, the morphine condition provides an important control for effects of morphine on heat pain sensitivity after capsaicin administration.

The facial testing paradigm in the present study can be regarded as a pain tolerance test, meaning that tolerance for heat pain is increased by the conflict between escape and feeding. This likely accounts for differences found between effects of low dose systemic morphine on escape with and without the conflict with food reinforcement. Systemic morphine (0.5 mg/kg) has been shown to attenuate escape from heat stimulation of the paws of rats on an operant paradigm that does not include food reward [21]. This comparison with the present study suggests that the feeding conflict increases tolerance for low levels of heat pain in the naïve condition but does not overcome suprathreshold pain elicited by the combination of capsaicin and heat stimulation. For the food conflict paradigm, enhancement of C nociceptor input is required to reveal effects of low dose morphine.

When animals were tested at a cool temperature (24 °C), several outcome measures were higher following capsaicin treatment, as compared to naïve animals. Both reward intake (Fig. 1A) and facial contact duration (Fig. 1B) were significantly lower in normal animals, while licking contacts and the ratio of facial duration/contact were insignificantly lower for naïve animals. Taken together, this indicates that the cool temperature has a soothing or inhibitory effect against the burning sensation associated with capsaicin. Activation of the TRPM8 receptor via cool temperatures [12,15] may produce a counteracting or inhibitory effect against TRPV1-mediated responses. Exploration of this mechanism through use of this thermal operant assay may lead to significant insights regarding mechanisms underlying both heat and cold pain modulation.

There was a significant decrease in response threshold to von Frey filament testing, indicating that peripheral application of capsaicin enhances both the responsivity of local reflex circuits and trigeminothalamic projection neurons to input from nociceptive afferents. Also, reduction by capsaicin of facial contact durations and the number of facial contact events at 38 °C indicates that there could be an influence of mechanical allodynia on the operant outcome measures. However, the results at 24 °C are not supportive, and additionally, the results from statistical analyses indicate that temperature had a significant and dominant impact on outcome measures following capsaicin treatment. Taken collectively, animals pressing their face against the thermode during operant testing did not produce a change in the outcome measures as a result of light touch/contact allodynia associated with capsaicin. Thermal allodynia following capsaicin application appears to have extended down to 38 °C.

In the reflex and operant assays, a decrease in withdrawal threshold or the operant response measures by capsaicin were likely due to enhancement of responses to afferent input, but interpretation of a hypoalgesic response is less clear-cut. In the case of stimulation with von Frey filaments, an increase in threshold has the appearance of hypoalgesia. However, if the animal is impaired to make this head withdrawal response, then this can provide the same endpoint (i.e., increased threshold, decreased responsivity). Morphine reversed the lowering of von Frey thresholds produced by capsaicin; however, head withdrawal responses of untreated animals were also significantly suppressed following morphine administration. Thus, morphine could have attenuated facial motoneuronal responses to mechanical stimulation after capsaicin. In contrast morphine attenuated capsaicin-induced allodynia/hyperalgesia in the operant assay at a dose which did not affect the same motor response in the absence of capsaicin. This point is particularly relevant when considering that the management of pain involves a balance between inhibition and suppression of pain and minimization of untoward side effects. Additionally, one must evaluate and consider general dose limitations relating to sedation/side effects prior to choosing the appropriate analgesic dose for whatever drug is being studied. We purposely chose the 0.5 mg/kg dose of morphine because this dose does not significantly affect general behavior.

Investigations of new analgesic treatments ideally are coupled with the use of compassionate methods for pain testing in animals. This study demonstrates that this is possible using the thermal operant orofacial assay in conjunction with capsaicin cream, to provide a reproducible, sensitive, and powerful approach for studying thermal pain. We demonstrated that both

heat hyperalgesia and allodynia produced with a low dose of capsaicin can be detected and quantified using this assay. This is significant because this was obtained with a mild stimulus that does not produce permanent impairment or tissue damage. The establishment of this reproducible facial pain system provides a non-invasive, quantifiable assessment of trigeminal pain conditions that models the human pain experience, taking into consideration the effects of higher level cognitive processing. This provides a pivotal link for translating basic pain research into clinic trial strategies.

Acknowledgements

This work was supported by Grant #1K22DE014865-01A1 (National Institute of Dental and Craniofacial Research, National Institutes of Health, Department of Health and Human Services, Bethesda, MD, USA), the University of Florida, College of Dentistry, and the Evelyn F. and William L. McKnight Brain Institute.

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