Bite Force Measurement in Awake Rats: A Behavioral Model for Persistent Orofacial Muscle Pain and Hyperalgesia

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Aims: *To test the hypotheses that masseteric inflammation produces a reduction of mean bite force and success rates and that classical anti-inflammatory agents prevent inflammation-induced changes in bite force.* ***Methods:*** *Rats were initially trained to produce a bite force greater than 400 g. Once the rats attained above 70% of successful responses in a 10-minute test period, the bite force required for reinforcement was increased gradually to the target force of 1.3 kg. Seven trained rats received bilateral masseteric injections of complete Freund's adjuvant (CFA; 50 µL in isotonic saline). The mean number of attempted bites, the percentage of correct responses, and the bite force measured before and 1, 2, 3, 7, 10, and 14 days following the CFA injection were compared. Five additional trained rats were injected with the same volume of vehicle control. Other rats (n = 10) were treated with anti-inflammatory agents before and after the CFA injection.* ***Results:*** *Intramuscular CFA, but not the vehicle, produced a significant reduction of mean bite force and success rate at days 1, 2, and 3. Bite force and success rate gradually increased; they returned to baseline by 14 days. The CFA-mediated reduction of bite force and success rate was prevented in rats treated with anti-inflammatory agents administered intraperitoneally (dexamethasone, n = 5, or indomethacin, n = 5, 4 mg/kg).* ***Conclusion:*** *These results provide further evidence that bite force measurements in awake rats can be a useful method for the study of inflammatory muscle hyperalgesia.* J OROFAC PAIN 2005;19:159–167

Key words: bite force, masseter inflammation, rat behavior

Chronic musculoskeletal pain is a significant health problem that affects a large percentage of the population. However, the etiology and pathophysiology of chronic muscle pain are still largely unknown. One of the limitations of studying the underlying mechanisms of persistent pain arising from muscle tissue has been the lack of animal models that can be used to assess muscle pain conditions. Development of animal models of muscle pain is complicated because persistent muscle pain is associated with alterations in both deep and cutaneous tissue sensitivity as well as muscle function. Persistent muscle pain, including that originating from masticatory muscles, is characterized by localized pain in the muscle, referral to other areas, tenderness upon palpation, restricted range of movements, and reduced maximum force generated by the muscle.[1,2] Thus, behavioral models that

assess only the changes in cutaneous sensitivity following the induction of muscle pain have limited applicability to the study of clinical muscle pain conditions.

The relationship between muscle function or dysfunction and pain has been a topic of study for many decades.[3–5] Lund et al[3] suggested that pain reduces the output of a muscle when acting as an agonist and increases its activity when acting as an antagonist. In accordance with these predictions, previous clinical studies have provided ample evidence that muscle output in chronic pain patients suffering from different types of muscle disorders is significantly reduced.[2,3,6,7] Induction of experimental muscle pain in healthy human subjects causes a significant reduction of masseter electromyographic (EMG) activity when the muscle is acting as an agonist during mastication.[8] These observations suggest that force output in the presence of muscle pain and inflammation may serve as a useful measure of muscle pain and hyperalgesia.

Kehl et al[9] introduced an animal model that measures forelimb muscle force in rats. Consistent with observations from human studies, they showed that experimentally induced myositis evokes a time- and dose-dependent reduction in forelimb grip force that can be reversed by classical anti-nociceptive and anti-inflammatory pharmacologic agents. These results have provided strong evidence for the validity of the use of inflammation-induced muscle force reduction as an index of muscle hyperalgesia in animal studies. However, an equivalent animal behavioral model for the study of persistent orofacial muscle pain has not been available. The author recently described a simple and reproducible behavioral paradigm to measure bite force in a rodent model.[10] It was demonstrated that rodents can be trained to produce a specific bite force and that the new analysis techniques introduced offer a flexible and effective means for monitoring and analyzing bite force. In the present study, this model was utilized to test the hypotheses that masseteric inflammation produces reduction of mean bite force and success rates, and that classical anti-inflammatory agents prevent the inflammation-induced changes in bite force.

Materials and Methods

Subjects

Twenty-two male Sprague-Dawley rats, weighing between 300 and 350 g, were trained to produce a specific bite force. They were housed in separate cages in standard conditions with 12-hour light:12-hour dark cycles. All procedures in this study were conducted within the U.S. National Institutes of Health guidelines for Care and Use of Laboratory Animals under a University of Maryland–approved Institutional Animal Care and Use Committees protocol.

Apparatus and Training Procedure

The apparatus and training procedures have been described previously.[10] Briefly, the components of the system used to measure bite force from the awake rats consisted of a strain-gauge force transducer (Model FT03, Grass Instruments) attached to a pair of custom-made brass bite plates and CED 1401+ (Cambridge Electronics Design) interfaced to a Pentium III personal computer. The strain gauge was calibrated daily for forces between 0 and 2,000 g. In between the bite plates was placed PE90 tubing, which was connected to an electronic water delivery system (Crist Instrument) that was directly presented to the rat's mouth. The analog signal from the force transducer was sampled and digitized by the CED 1401+ through a Spike 2 script program (CED). The script program detects the threshold crossing on a bite-force signal channel online and sends a transistor-transistor logic (TTL) pulse to an external device upon detection. Thus, when bite force reaches the threshold, the TTL pulse triggers a contact closure of the water delivery system, which results in the rat receiving 0.03 mm^3 of water as reinforcement.

The rats were maintained on a water-restriction schedule during the course of the experiment to maintain a body weight not less than 80% of the average body weight of nontraining rats, who were fed ad libitum. Water intake was closely regulated to ensure that animals remained healthy but were motivated to perform the behavioral task. Rats were initially trained to produce bite forces greater than 400 g, since bites of lower forces were difficult to distinguish as discrete bites.[11] This force level was designated the "cutoff" force. Bite forces that were less than the cutoff force were not included in the analysis; only bites whose force was greater than the cutoff force were considered as attempted trials.

Once the rats learned to bite with forces greater than 400 g, they were trained to produce a gradually increasing target force and allowed to bite as much as they could in a 10-minute session. Success rate was determined as the ratio between the number of rewarded bites (only those exceeding a target force) and the total number of attempted bites (the number of all bites with forces greater than

the cutoff force). When a success rate of greater than 70% was attained, the rats were moved to a higher force, until they reached the final target force of 1.3 kg. A final target force of 1.3 kg was chosen for this study because rats can be trained to produce this level of force within 3 to 4 weeks.[10]

Induction of Muscle Inflammation and Drug Injections

Once the rats reached the target force of 1.3 kg with a greater than 70% success rate for 3 consecutive sessions, they were assigned to 1 of the following experimental or control groups. The first group of rats (n = 7) received bilateral injections of a small volume of an inflammatory agent, complete Freund's adjuvant (CFA, 50 μL; 1:1 isotonic saline) in the midregion of the masseter muscle. The second group of rats (n = 5) received the same volume of vehicle control injections (isotonic saline) in the same manner. Animals were briefly anesthetized with 1% to 2% halothane for intramuscular injection procedures. To determine if treatment with anti-inflammatory drugs could block the effects of CFA on the bite task, additional rats were treated with either a steroidal anti-inflammatory drug (4 mg/kg dexamethasone administered intraperitoneally; n = 5) or a non-steroidal anti-inflammatory drug (4 mg/kg indomethacin administered intraperitoneally; n = 5) 4 hours prior to the CFA injection, 24 hrs afterward, and 48 hrs afterward.

Data Analysis

Three dependent variables, the total number of attempted bites, success rate, and mean bite force (mean of all attempted bites within a 10-minute session) were collected before the CFA injection and compared to those collected 1, 2, 3, 7, 10, and 14 days following the CFA injection. Measurements for each dependent variable collected during 3 preinjection sessions were averaged and used as the baseline value. Postinjection measurements were normalized to the baseline value and percent change from the baseline was calculated and plotted against time. The percent change for each dependent measurement from the baseline for all groups was analyzed by 1-way repeated-measures of analysis of variance (ANOVA). Post hoc comparisons were performed with Dunnett's test. The significance level was set at $P < .05$.

Results

General Observations

All animals successfully learned to acquire water reinforcement by producing vertical bites on the brass bite plates. Initially, the rats exhibited extensive exploratory behaviors, but gradually they learned to associate the bite with water reinforcement. They learned to exceed the cutoff force (400 g) within 5 training sessions and the final target force within 3 to 4 weeks. As previously noted,[10] both mean success rate and mean bite force ± SD measured reliably across the rats (75.1% ± 4.2% and 1,375 ± 48.6 g, respectively).

Figure 1 shows examples of bite responses collected before the injection procedures from 2 different rats. The upper 2 panels were taken from a rat that produced a high number of total bites. This rat responded with 1 or 2 distinct bites all throughout the task. Another rat, whose data are shown in the bottom 2 panels, produced a lower number of total bites but attained the pattern of multiple bites for each biting episode. In both cases, bites that did not meet the cutoff force were not included in the analysis, and bites that did not reach the target force were not rewarded and were not considered as successful trials.

Effects of Muscle Inflammation on Bite-Force Behavior

Intramuscular injections with CFA significantly altered the behavioral measures in a time-dependent manner. Figure 2a shows the changes in success rate of both CFA- and vehicle-injected rats. Intramuscular injection of CFA, but not the vehicle, produced a significant reduction of percentage of correct responses at days 1, 2, and 3 following the injection ($F = 7.4$; $P < .001$). Maximum success rate reduction was observed 24 hours following the CFA injection. The success rate gradually increased; it returned to baseline by 14 days postinjection.

Since assessment of the success rate does not reveal the magnitude of force reduction following the myositis, mean bite force was also analyzed. A significant and reliable reduction in mean bite force was also seen in CFA-injected rats (Fig 2b; $F = 5.661$; $P < .001$) but not in vehicle-treated rats. The CFA injection consistently yielded greater than 15% reduction of the mean bite force from the preinjection level 24 hrs following the injection. However, the total number of trials was not

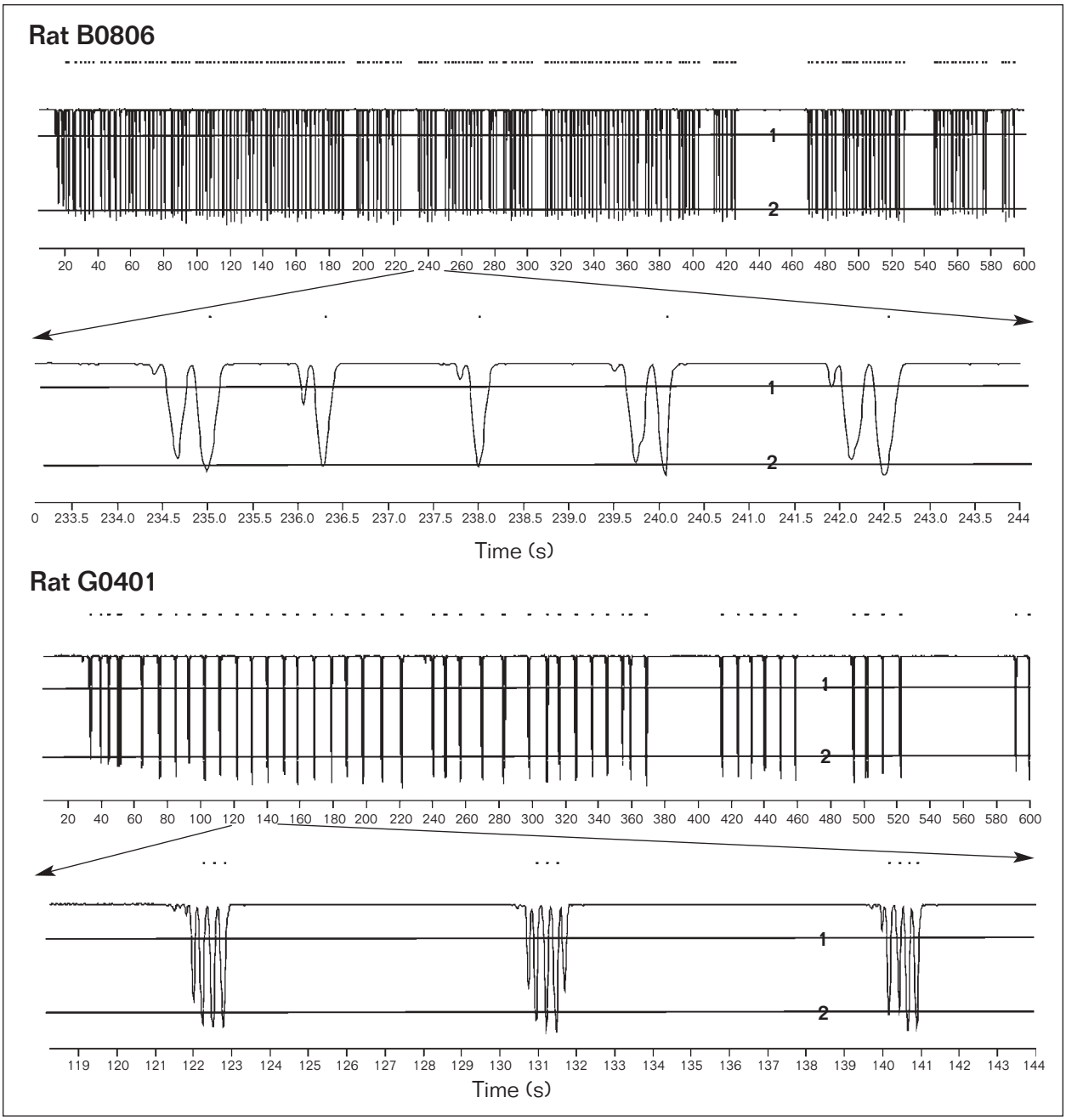

Fig 1 Screen views taken from the analysis script program for 2 different rats. For each rat, the top screen view shows the bite-force data from a 10-minute session. The horizontal line labeled *1* represents the cutoff force level of 400 g; the horizontal line labeled *2* represents the target force level of 1.3 kg. The dots on top represent the rewarded trials. The lower screen views show an expanded view of individual bite attempts. The rats exhibited different bite patterns. Only bites that crossed the target force line (line 2) were rewarded. During the analysis, only bites that crossed the cutoff force (line 1) were considered discrete bites.

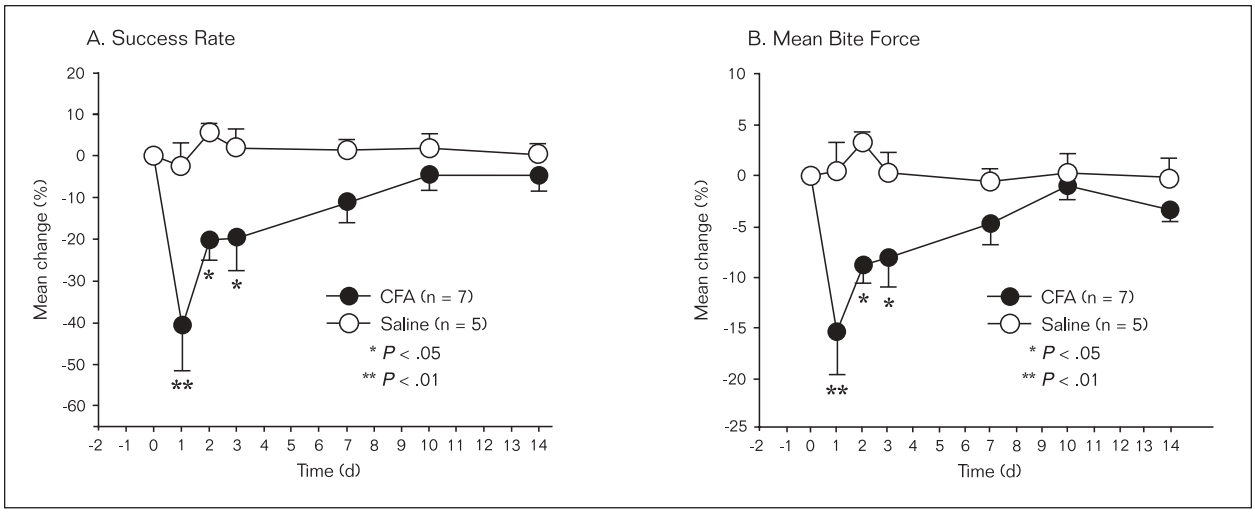

Fig 2 Time-course of the changes in bite-force parameters, success rate *(a)* and mean bite force *(b)*, following masseter injections with CFA. Intramuscular injections with CFA significantly reduced the mean success rate and mean bite force on days 1, 2, and 3 following the CFA injections. Isotonic saline injections produced no significant changes. For this figure and subsequent figures, asterisks indicate significant differences (* $P < .05$, ** $P < .01$) from the preinjection level, and error bars in each graph represent SEM.

Table 1 Percent Weight Changes After CFA or Isotonic Saline Injection

Treatment	No. of animals treated	No. of days postinjection					
		1	2	3	7	10	14
CFA	7	–1.04 ± 1.11	–1.66 ± 1.22	–1.95 ± 1.95	1.02 ± 2.33	–0.94 ± 2.26	0.79 ± 3.80
Saline	5	–1.96 ± 2.48	–2.13 ± 2.18	–3.69 ± 2.38	0.84 ± 4.58	0.66 ± 3.68	1.24 ± 3.69

Data shown is for animals not treated with anti-inflammatory drugs.

significantly affected by the injection procedures. A greater variability was associated with the total number of trials among the rats, and neither the CFA nor the vehicle injections caused statistically significant changes in the total number of trials from the baseline levels ($F = 0.916$, $P > 0.49$ for CFA; $F = 0.637$, $P > .7$ for vehicle).

Since it is possible that CFA-induced muscle inflammation could alter eating habits of the animals and cause a significant weight loss that could lead to bite-force reduction, the weight changes of both CFA- and vehicle-treated rats were closely monitored (Table 1). There was no significant weight change following either the CFA or vehicle injection procedure, indicating that the reduction of bite force was not associated with weight loss due to muscle inflammation. Also, bite-force reduction was not associated with an altered pattern of biting behavior. All rats were trained to produce only vertical bites and exhibited consistent bite behaviors throughout the experimental procedures.

Effects of Anti-inflammatory Drugs on CFA-Induced Bite Force Behavior

In order to confirm that the reduction of success rate and bite force was due to CFA-mediated inflammatory responses, separate groups of rats trained under the same experimental paradigm received treatments with anti-inflammatory drugs as described in the Materials and Methods section. The bar graphs shown in Fig 3 compare the mean percent changes in success rate and bite force from the preinjection levels for rats that received CFA alone and the rats that received CFA with either dexamethasone or indomethacin. Both dexamethasone and indomethacin substantially reversed the CFA-mediated reductions of mean success rate and mean bite force. The drug treatments were effective in blocking the CFA-mediated responses on days 1, 2, and 3 following the CFA, with the peak effect observed on day 2.

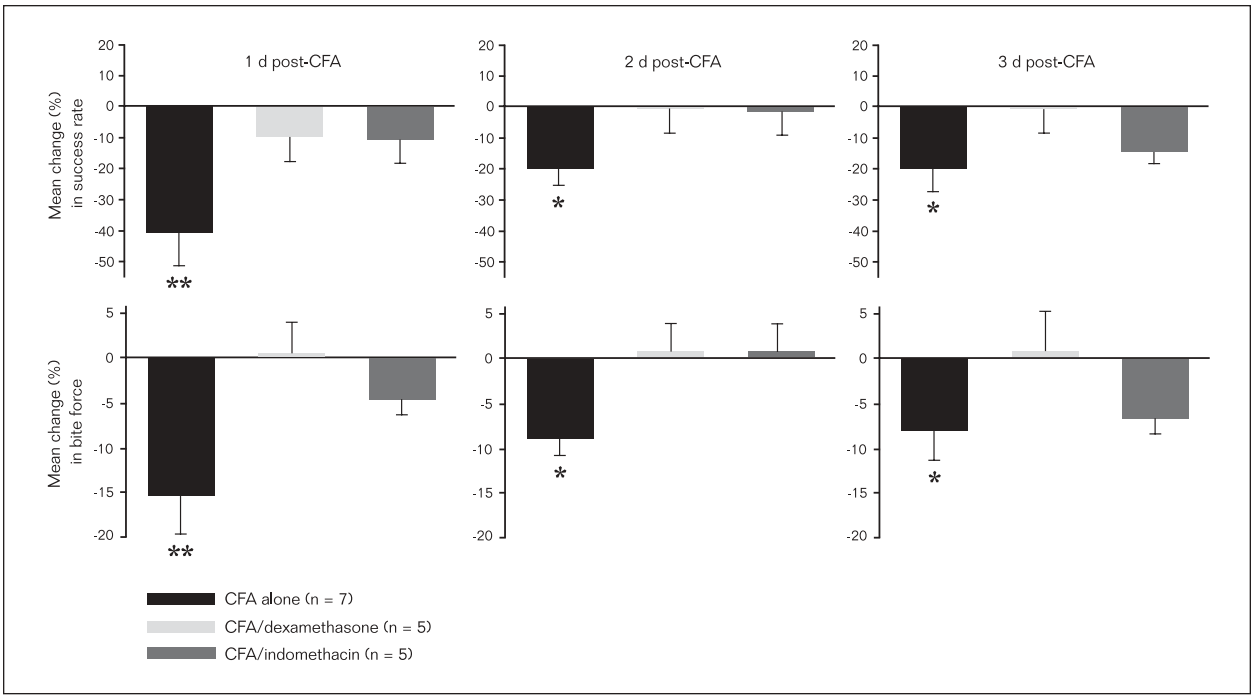

Fig 3 Effects of anti-inflammatory drugs on CFA-mediated bite force and success rate reduction. Rats treated with either dexamethasone or indomethacin did not exhibit significant reduction of mean success rate or bite force 1, 2, or 3 days following intramuscular injection of CFA.

Discussion

Muscle Force Reduction and Hyperalgesia

Muscle pain differs from cutaneous pain in subjective quality, accuracy of localization, and referral to other tissues.[4] A majority of central neurons receiving high-threshold muscle afferent input also process additional input from cutaneous structures.[12–14] Experimental myositis produces an alteration in the size and mechanical sensitivity of both muscle and cutaneous receptive fields.[13,15–17] Thus, the somatosensory effects of muscle pain and inflammation involve changes in deep as well as cutaneous tissue sensitivity. Behaviorally, the cutaneous hypersensitivity is reliably demonstrated by the findings of a significant reduction of withdrawal thresholds of the hindpaw following intramuscular injection of acidic saline, capsaicin, or carrageenan.[18–20] While the hindpaw withdrawal behavioral paradigm following cutaneous stimulation in the presence of muscle pain is useful to study secondary mechanical hyperalgesia, it has limited applicability to the study of the underlying mechanisms of primary muscle hyperalgesia or allodynia that are more frequently associated with clinical muscle pain conditions.

In the past, clinical and experimental studies have consistently demonstrated that patients with muscle pain show lower levels of force during maximum contraction compared to healthy subjects.[2–5] However, the causal relationship between muscle pain and reduced force output generated by the muscle has not been sufficiently demonstrated. The present study was designed with the assumption that muscle pain and inflammation reduce muscle force. A basis for the hypothesis that the reduction of bite force is correlated with masseter muscle hyperalgesia was provided by findings from a grip force reduction test in awake rats, which was created as a model for the study of primary hyperalgesia of inflamed muscles.[9] The validity of this model as an effective tool for the study of muscle hyperalgesia was demonstrated by a time-dependent reduction of force that was correlated with inflammatory responses. Classical anti-nociceptive agents significantly reversed the carrageenan-evoked grip-force reduction. Furthermore, capsaicin pretreatment prevented carrageenan-mediated grip-force reduction, suggesting that the force reduction was mediated by small-diameter primary afferents. The model has since been used to evaluate a variety of mechanistic hypotheses of muscle hyperalgesia.[21–23]

The reduction of bite force following muscle inflammation can be explained by the findings that mechanical stimuli such as muscle contraction and movement can begin to activate group III and IV fibers, which have a lowered threshold due to peripheral sensitization in the presence of muscle inflammation.[24,25] Afferents in limb muscles implicated in sensations of force and tension have conduction velocities similar to those of nociceptive afferents.[4] However, they are also clearly activated by non-noxious stimuli, including muscle contraction and stretching.[26,27] It is possible that these afferents maintain subthreshold postsynaptic connections to the dorsal horn neurons that reach the activation threshold as a result of central sensitization.[16,28–30] High-threshold muscle afferents that are activated during jaw muscle contraction can inhibit masseter motoneurons on both sides.[31] These observations, along with electrophysiological studies that showed the inhibition of gamma motoneurons by small-diameter muscle afferents following experimental myositis,[32] may provide neural substrates for force reduction in inflamed muscles.

In the present study, it has been shown that the CFA-mediated bite-force reduction changed in concert with the progression of CFA-induced inflammation. Bite force returned to the preinjection level in 14 days, and treatment with anti-inflammatory agents significantly reversed the CFA-mediated bite-force reduction. It is possible that the impairment of muscle performance is caused by disruption of muscle fibers by CFA injections. However, the results of the present study show that this is likely not the case. The observations that anti-inflammatory treatments effectively prevent CFA-induced bite force reduction and that bite force returns to the preinjection level with the passage of time suggest that the reduction in bite force is primarily mediated by pain and inflammation of the muscle. The reduction of mean bite force or success rate was not caused by lack of motivation of the rats following the CFA injection, since the total attempted trials were not significantly affected by the CFA injection. Thus, the results of the present study provide further validation of force reduction as a useful index of primary muscle hyperalgesia.

Behavioral Models for Orofacial Deep Tissue Pain

Currently, there are few animal behavioral models available to study orofacial deep tissue pain and inflammation. Roveroni et al[33] have recently introduced a behavioral model for temporomandibular joint (TMJ) pain in which rubbing of the orofacial region, mandibular rotation, and head flinching were characterized as formalin-induced behavioral responses. While the study validates the facial grooming behavior as a useful index for TMJ pain, its practical value in assessing the underlying mechanisms of TMJ pain is yet to be tested. Reflex EMG activity from masticatory muscles has also been used as an index of nociceptive responses following acute TMJ inflammation in anesthetized rats.[34] This model has served to elucidate the involvement of a variety of central and peripheral mechanisms in TMJ pain.[35–38] However, the anesthetized preparation does not permit the study of persistent changes in EMG activity under chronic pain conditions. Clinical studies suggest that the level of postural EMG activity may be no higher than normal in chronic musculoskeletal pain conditions.[3] Also, inflammation in the TMJ and masseter muscle may produce different EMG and behavioral responses.[33,39] Thus, it is important to develop an accurate assessment of nocifensive behavior resulting from the muscle tissue stimulation in order to study the tissue-specific underlying mechanisms.

The authors have recently introduced a new behavioral model for assessment of acute craniofacial muscle pain in lightly anesthetized rats.[40] The model utilizes stereotypical hindpaw-shaking behavior following algesic chemical stimulation of the muscle as an index of muscle nociception. A major advantage of this lightly anesthetized rat model is that it permits standard and reliable manipulation of experimental conditions and drug administration while assessing a quantifiable behavioral response.[41] One of the limitations of the model, however, is that it does not allow the measurement of inflammation-induced changes in muscle tissue sensitivity on a more long-term basis (eg, over a period of days). The present study has demonstrated that inflammation-induced bite-force changes can be reliably measured in behaving rats for a more prolonged period of time. Also, the bite force model provides a measure of primary hyperalgesia that is functionally relevant to clinical conditions. This model complements the assessment of acute nociceptive behavior by the hindpaw-shaking model, and enables us, for the first time, to study the mechanistic aspects of the development and maintenance of persistent orofacial muscle pain and hyperalgesia in awake animals.

Technical Considerations

Assessment of bite force in the awake rat is potentially important for the investigation of the under-

lying mechanisms of a variety of clinical conditions in which bite force is compromised. The simplified techniques presented here provided easier implementation of bite-force measurements for the development of a behavioral model of persistent orofacial muscle pain. However, a certain amount of training time is required for the animal to acquire the desired level of success. Target forces greater than 1.3 kg will require a longer training period. However, target forces less than 1 kg may not be sufficient to yield a robust change upon experimental manipulation. Data from the present study showed that CFA reduced the mean bite force by 15% on the first day. A more robust reduction of mean bite force is expected with a higher target force.

The interactive data capture apparatus and analysis software instruments used in this study can be modified to meet the specific needs of the user, but require a basic understanding of script language. The data capture apparatus allows online control of threshold bite force at anytime during the training session. The script program allows users to specify cutoff and target forces and the duration of data sample. The analysis instrument automatically computes parameters such as maximum value and the time of the maximum value. At the end of the analysis, all dependent measures are automatically calculated and stored as a log file. Additional parameters such as bite pattern and bite duration, as shown in Fig 1, can also be analyzed with the relatively simple addition of instructions in the script program.

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