Nerve growth factor alters the sensitivity of rat masseter muscle mechanoreceptors to NMDA receptor activation

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1Faculty of Pharmaceutical Sciences, The University of British Columbia, Vancouver, British Columbia, Canada; 2College of Stomatology, Tianjin Medical University, Tianjin, China; and 3Center for Sensory Motor Interaction, Department of Health Science and Technology, Faculty of Medicine, Aalborg University, Aalborg East, Denmark

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Wong H, Dong XD, Cairns BE. Nerve growth factor alters the sensitivity of rat masseter muscle mechanoreceptors to NMDA receptor activation. J Neurophysiol 112: 2275–2282, 2014. First published August 13, 2014; doi:10.1152/jn.00327.2014.—Intramuscular injection of nerve growth factor (NGF) into rat masseter muscle induces a local mechanical sensitization that is greater in female than in male rats. The duration of NGF-induced sensitization in male and female rats was associated with an increase in peripheral N-methyl-D-aspartate (NMDA) receptor expression by masseter muscle afferent fibers that began 3 days postinjection. Here, we investigated the functional consequences of increased NMDA expression on the response properties of masseter muscle mechanoreceptors. In vivo extracellular single-unit electrophysiological recordings of trigeminal ganglion neurons innervating the masseter muscle were performed in anesthetized rats 3 days after NGF injection (25 μg/ml, 10 μl) into the masseter muscle. Mechanical activation threshold was assessed before and after intramuscular injection of NMDA. NMDA injection induced mechanical sensitization in both sexes that was increased significantly following NGF injection in the male rats but not in the female rats. However, in female but not male rats, further examination found that preadministration of NGF induced a greater sensitization in slow Aδ-fibers (2–7 m/s) than fast Aδ-fibers (7–12 m/s). This suggests that preadministration of NGF had a different effect on slowly conducting mechanoreceptors in the female rats compared with the male rats. Although previous studies have found an association between estrogenic tone and NMDA activity, no correlation was observed between NMDA-evoked mechanical sensitization and plasma estrogen levels. This study suggests NGF alters NMDA-induced mechanical sensitization in the peripheral endings of masseter mechanoreceptors in a sexually dimorphic manner.

nerve growth factor; masseter muscle; mechanoreceptors; sensitization; NMDA receptors

GLUTAMATE IS THE MAJOR EXCITATORY neurotransmitter in the central nervous system (Platt 2007). Evidence suggests that it may also play a role in peripheral nociception (Carlton et al. 1995; Haas et al. 2010; Lam et al. 2005; Miller et al. 2011). Glutamate and its receptors are found in trigeminal and dorsal root ganglion neurons and at their central and peripheral terminals (Cairns et al. 2003a; Dong et al. 2007; Li et al. 2004; Wong et al. 2014). Peripheral glutamate levels are elevated during cutaneous or deep tissue inflammation, produced by a variety of nonneuronal cells such as mast cells and macrophages (Newsholme and Calder 1997). Glutamate may also be released by the peripheral endings of the primary afferent fibers during nociceptive stimulation and acts on the glutamate receptors on the primary afferent fibers themselves (deGroot et al. 2000; Westlund et al. 1989). This raises the possibility that peripheral glutamate may have an autocrine and/or paracrine role in a positive-feedback enhancement of nociceptor excitability (Lam et al. 2005).

A number of clinical and animal studies have provided behavioral evidence in support of a role for peripheral glutamate receptors in the transduction of masticatory muscle nociceptive signaling. In particular, glutamate concentrations were significantly higher in the masseter muscles of myofascial temporomandibular disorder (TMD) patients compared with healthy controls (Castrillon et al. 2010). Injection into the masseter muscle of healthy subjects induced pain and mechanical sensitization, and this response was blocked by an N-methyl-D-aspartate (NMDA) receptor antagonist (Svensson et al. 2003). Glutamate-evoked pain was also found to be greater in women than men, similar to the sex-related difference in the occurrence of TMD pain (Cairns and Gazerani 2009). In rat masseter muscle, baseline interstitial glutamate concentration was found to be ~25 μM, and increasing this concentration two to three times via systemic injection of monosodium glutamate resulted in a decrease in the mechanical activation threshold (MT) of masseter muscle nociceptors (Cairns et al. 2007). These results suggest that masseter muscle afferent mechanical sensitivity may be regulated, in part, through peripheral NMDA receptors.

The neurotrophin NGF has recently been found to be an important peripheral mediator of nociception. Elevated levels of NGF have been found in many chronic pain conditions (Anand 1995). Injection of NGF into the masseter muscle of healthy subjects resulted in a local mechanical sensitization that lasted for 1–3 wk, with a greater magnitude in women than men (Svensson et al. 2003, 2008a). In a previous study, we demonstrated that injection of NGF into the rat masseter muscle induced a prolonged local mechanical sensitization, which was also greater in female rats than male rats (Wong et al. 2014). The duration of this sensitization was associated with an increased expression of NMDA receptors in masseter muscle afferent fibers, suggesting that NGF-induced sensitization may be maintained by increased peripheral NMDA receptor expression.

In the present study, we examined whether preadministration of NGF alters the response of masseter muscle afferent fibers to peripheral NMDA receptor activation in rats. We also examined whether this effect exhibits a sex-related difference. The results from this study indicate a significant interaction...
between NGF and the ability of peripheral NMDA receptors to modulate the mechanical sensitivity of masseter muscle afferent fibers.

METHODS

Animals. Male (310–480 g, n = 22) and female (230–300 g, n = 21) Sprague-Dawley rats were used for the experiments. Animals were housed in groups of two with a 12:12-h light-dark cycle. Food and water were given ad libitum. All animal procedures were reviewed and approved by the University of British Columbia Animal Care Committee.

Administration of NGF. Intramuscular injection of NGF (25 µg/ml, 10 µl; Sigma) or vehicle (10 µl; PBS; Sigma) was injected into the right masseter muscle after the animal was briefly anesthetized with isoflurane (2–2.5%; AErrane; Baxter, Mississauga, Ontario, Canada) and oxygen (97–98%). The experimenter was blinded to the content of the injections. The masseter muscle region was shaved before injection, and the injection site was marked with a permanent marker for subsequent identification. The concentration of NGF was selected based on the concentration used in previous human experimental pain studies and acute experiments in rats (Mann et al. 2006; Svensson et al. 2003, 2008a, 2010; Wong et al. 2014). Injections of NGF or saline were made 3 days before electrophysiological recording experiments in anesthetized rats.

Surgical preparation. Rats were anesthetized with isoflurane (2–2.5% in oxygen 97–98%; AErrane; Baxter). Blood pressure was monitored via a cannula inserted into the carotid artery and connected to a pressure transducer. A trachea tube was inserted to ventilate the animal throughout the experiment, and body temperature was maintained at 37.0 ± 0.2°C with an electric heating pad controlled from a rectal thermometer. The heart rate and blood pressure were monitored throughout the experiment. The hair of the right side of the face was shaved before the animal’s head was positioned in a stereotaxic frame. An incision was made to the skin over the dorsal surface of the skull to expose the skull bone, and a small trephination was made in the bone to allow lowering of a microelectrode through the brain into the trigeminal ganglion for recording. An incision was also made to the skin over the neck to expose the brain stem, and the dura was removed to allow a stimulating electrode to contact the brain stem.

Antidromic collision was performed to confirm projection of the masseter muscle fiber to the caudal brain stem. A stimulating electrode (parylene-coated tungsten microelectrode, 0.10 in., 2 MΩ; A-M Systems, Carlsborg, WA) was lowered into the ipsilateral caudal brain stem, and a constant-current electric stimulus (100-µs biphasic pulse, 10–90 µA, 0.5 Hz) was applied to evoke antidromic action potentials. Orthodromic action potentials were evoked by mechanical stimulation of the masseter muscle. Collision was demonstrated by disappearance of the antidromic spike (Fig. 1A). The straight line distance between the stimulating and recording electrodes was divided by latency of the antidromic action potential to estimate conduction velocity.

MT was assessed with an electronic von Frey hair (model 1601C; Life Science, Woodland Hills, CA). Mechanical stimuli were applied to the receptive field of the fiber at 1-min intervals for 10 min to obtain a baseline threshold (minimum force required to evoke afferent discharge; Fig. 1B). Force was increased gradually until a response was observed (Fig. 1B). After baseline recording, a 26-gauge needle connected to a 25-µl Hamilton syringe (Hamilton, Reno, NV) via a polyethylene tube that contained NMDA was inserted into the mechanoreceptive field of the masseter muscle afferent fiber. A 10-min recording period followed to record any baseline spontaneous discharge. After this baseline measurement, a single injection of NMDA...
were compared between the vehicle and NGF treatment groups by observed in the vehicle group of the male rats (coefficient previous study (Mann et al. 2006). However, a significant /H11002 0.776) but not in the female rats, which is consistent with a A

MT. MTs in the same animal before and after NMDA injection was used to determine the relationship between conduction velocity and MT. MTs in the same animal before and after NMDA injection were compared using a paired t-test. Relative MT (Rel MT) was calculated as post-NMDA MT/baseline MT × 100. MTs and Rel MTs were compared between the vehicle and NGF treatment groups by using a Student’s t-test. Two-way ANOVA on Rel MT with treatment and conduction velocity as factors was performed on fast and slow Aδ-fibers with post hoc Holm-Sidak multiple comparisons. Differences in the proportion of afferent fibers with spontaneous discharges before and after NMDA injection were compared between NGF and control treatment by using the Fisher exact test. The frequency of discharge (spikes per minute) after NGF and control treatments were compared with the Mann-Whitney rank sum test. A probability level of <0.05 was considered significant for all tests.

RESULTS

MT. Before NMDA injection, no significant difference was observed in the mean baseline MTs between the vehicle (n = 12) and NGF groups (n = 12) in male (vehicle, 30.5 ± 9.3 g; NGF, 32.3 ± 8.2 g) or female rats (vehicle, 34.2 ± 9.6 g; NGF, 41.9 ± 11.1 g). The baseline MTs were plotted against conduction velocities of the afferent fibers (Fig. 2). A significant inverse correlation between conduction velocity and MT was observed in the vehicle group of the male rats (coefficient = −0.776) but not in the female rats, which is consistent with a previous study (Mann et al. 2006). However, a significant inverse correlation (coefficient = −0.685) between conduction velocity and MT was found in the NGF-treated female rats. In Fig. 2, the lines represent masseter mechanical withdrawal thresholds determined from an earlier behavioral study in male and female rats at 3 days after intramuscular injections of vehicle or NGF into the masseter muscle (Wong et al. 2014). The injection procedure (vehicle, NGF concentration, and injection volume) in that study was identical to the one employed here. Afferent fibers with MTs above the respective line (vehicle or NGF treatment) were considered to be putative mechanonociceptors. The number of masseter afferent fibers with a MT above the withdrawal threshold was found to be 3/12 in the vehicle group and 5/12 in the NGF group in the male rats, whereas a bigger difference was found between the vehicle (3/12) and NGF groups (7/12) in the female rats. None of the differences reached statistical significance (Fisher exact test, P > 0.05).

NMDA injection significantly reduced MT in all treatment groups compared with the baseline MT in male (vehicle, 25.4 ± 8 g; NGF, 21 ± 7.1 g) and female rats (vehicle, 27.7 ± 7.9 g; NGF, 30.7 ± 10.3 g). In the male rats, NMDA-induced mechanical sensitization was significantly greater in the NGF group than the vehicle group (Fig. 3, A and B). In female rats, NMDA-induced mechanical sensitization was increased in the NGF group, however, the difference was not significant (P > 0.05). The relationship between conduction velocity and Rel MT is presented in Fig. 3, C and D. The scatterplot shows that in female rats, NGF appeared to enhance NMDA-induced mechanical sensitization primarily for Aδ-
fibers with slower conduction velocities. Further analysis was
carried out by separating the two populations based on con-
duction velocity. They were separated at the midpoint of the
conduction velocity range of the Aδ-fiber into slow Aδ-fiber
(2–7 m/s) and fast Aδ-fiber (more than 7–12 m/s) groups.
Two-way ANOVA on Rel MT with treatment and conduction
velocity as factors was performed with post hoc Holm-Sidak
multiple-comparisons test. In the female rats, there was a
significant interaction between vehicle and NGF in the fast-conduction velocity group. In the male rats, there was a
significant effect of treatment but no significant effect of conduction velocity or interaction between treatment and conduction
velocity. Post hoc testing indicated a significant difference
between vehicle and NGF in the fast-conduction velocity group.
In the female rats, there was a significant interaction between
treatment and conduction velocity, but there was no significant
effect of treatment or conduction velocity. Post hoc testing indicated a significant difference between slow- and fast-
conduction velocity groups in the NGF groups.

Plasma estrogen concentration of the female rats was determined since systemic estrogen has been shown to modulate
the sensitivity of masorset afferent fibers to NMDA (Dong et al.
2007). The plasma concentration of estrogen was not different
between the vehicle (63.3 ± 14.5 pg/ml) and the NGF (70.8 ±
20 pg/ml) groups (Student’s t-test, P > 0.05). No significant
correlation was observed between plasma estrogen concentra-
tion and baseline MT or Rel MT (Fig. 4).

Spontaneous and NMDA-evoked discharge. The numbers of
spontaneous discharges and NMDA-evoked discharges were
evaluated over a 10-min period before and after NMDA injec-
tion. No difference in the proportion of afferent fibers with
spontaneous discharges before and after NMDA injection was
found between the vehicle and NGF treatment groups in either
sex (Table 1). NGF treatment also had no effect on the proportion
of afferent fibers with spontaneous discharges and NMDA-evoked discharges was
examined for the NGF treatment group is plotted against baseline MT
and Rel MT (Fig. 5, B and C). No correlation was observed,
demonstrating that the distance between NGF injection and
the field of the recorded fiber (Fig. 5). To determine whether
this distance affected the results, the distance between the
initial NGF injection and the subsequent NMDA injection was
plotted against baseline MT and Rel MT (Fig. 5, B and C). No correlation was observed,
demonstrating that the distance between NGF injection and
the subsequent NMDA injection was
plotted against baseline MT and Rel MT (Fig. 5, B and C). No correlation was observed,
demonstrating that the distance between NGF injection and

Distance. Since the masorset fibers were randomly iden-
tified through mechanical stimulation of the masorset muscle,
one possible confounding factor could be the distance
between the initial NGF injection and the mecanorperceptive
field of the recorded fiber (Fig. 5A). To determine whether
this distance affected the results, the distance between the
initial NGF injection and the subsequent NMDA injection
for the NGF treatment group is plotted against baseline MT
and Rel MT (Fig. 5, B and C). No correlation was observed,
demonstrating that the distance between NGF injection and
the subsequent NMDA injection was
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demonstrating that the distance between NGF injection and
the subsequent NMDA injection was

Fig. 3. A and B: relative mean MT (Rel MT) of masorset mecanororeceptors (n = 12) 10
min after intramuscular N-methyl-D-aspartate (NMDA) injection in male and female
rats. Rats were treated with NGF or vehicle 3 days before NMDA injection. Rel MT =
postinjection MT/baseline MT × 100. *P < 0.05, Student’s t-test. C and D: the scatter-
plot shows the relationship between Rel MT and conduction velocity of masorset me-
canororeceptors in male and female rats. E and F: comparison between the Rel MT of slow
Aδ-fibers (conduction velocity: 2–7 m/s) and fast Aδ-fibers (conduction velocity: 7–12
m/s) after vehicle or NGF treatment in male and female rats. Only Aδ-fibers (conduction
velocity: 2–12 m/s) were included in the analysis. Male (slow: vehicle, n = 3; NGF,
n = 4, fast: vehicle, n = 5; NGF, n = 7). Female (slow: vehicle, n = 5; NGF, n = 6,
fast: vehicle, n = 5; NGF, n = 5), *P < 0.05, 2-way ANOVA and post hoc Holm-Sidak
multiple-comparisons test.
Table 1. The proportion of masseter afferent fibers with spontaneous discharge before and after NMDA injection

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Before NMDA</th>
<th>Male</th>
<th>Female</th>
<th>After NMDA</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>6/12</td>
<td>4/12</td>
<td>4/12</td>
<td>6/12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NGF</td>
<td>4/12</td>
<td>4/12</td>
<td>5/12</td>
<td>6/12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

No significant difference was observed between vehicle and NGF groups before and after NMDA injection (Fisher exact test \( P > 0.05 \)).

Table 2. The mean discharge rate in masseter afferent fibers before and after NMDA injection

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Before NMDA, Spikes/min</th>
<th>Male</th>
<th>Female</th>
<th>After NMDA, Spikes/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0.7 ± 0.4</td>
<td>0.6</td>
<td>0.4</td>
<td>0.9 ± 0.6</td>
</tr>
<tr>
<td>NGF</td>
<td>0.8 ± 0.7</td>
<td>0.4</td>
<td>0.2</td>
<td>1.7 ± 1.3</td>
</tr>
</tbody>
</table>

Values are means ± SE. The rate of spontaneous discharge was calculated from a 10-min period before or after NMDA injection. No significant difference was observed between vehicle and NGF groups before and after NMDA injection in either sex by Mann-Whitney rank sum test \( P > 0.05 \).

mechanical sensitization was associated with an increased expression of NR2B-subunit-containing NMDA receptors in masseter ganglion neurons in both male and female rats and was partly attenuated by an intramuscular injection of the NMDA receptor antagonist, 2-amino-5-phosphonovaleterate, 3 days after NGF injection, in the male rats (Wong et al. 2014).

In the present study, we examined whether NGF alters the response of individual masseter mechanoreceptors to peripheral NMDA receptor activation. NGF increased the level of mechanical sensitization in masseter muscle afferent fibers after local injection of NMDA into the masseter muscle, suggesting the effect of NGF occurs in the peripheral endings of muscle afferents. Taken together, these results suggest that a peripheral mechanism whereby NGF induces local mechanical sensitization by increasing the expression of NMDA receptors at the peripheral endings of masseter mechanoreceptors underlies the sensitization produced by NGF injections in rats at 3 days after NGF injection.

Intramuscular injection of NMDA has been shown to evoke discharges in masseter muscle mechanoreceptors, and increasing the expression of NMDA receptors by estrogen treatment has been shown to increase the magnitude of this discharge (Dong et al. 2007; McRoberts et al. 2007). Since we previously found NGF increases expression of NMDA receptors in masseter muscle ganglion neurons, we predict that a greater number of NMDA receptors would result in an increase in NMDA-evoked discharges (Wong et al. 2014). However, that was not the case, as NGF had no effect on the proportion of fibers with discharges or the magnitude of the discharges after NMDA injection. Interestingly, this result agrees with an earlier human pain experiment where preinjection of NGF had no effect on the intensity of glutamate-evoked masseter muscle pain in healthy humans (Svensson et al. 2008b). Furthermore, intramuscular injections of NGF into the masseter muscles of healthy men and women resulted in a prolonged (7- to 14-day) localized mechanical sensitization without reports of spontaneous pain (Svensson et al. 2003, 2008a). Here, we found that NGF increased NMDA-induced mechanical sensitization but had no effect on the frequency of spontaneous discharges. These results suggest that NGF does not cause spontaneous pain but may modulate mechanical sensitivity in putative masseter nociceptors by increasing the expression of peripheral NMDA receptors.

Previous human and rat behavioral experiments found that NGF reduced mechanical thresholds after intramuscular injection into the masseter muscle (Svensson et al. 2003, 2008a; Wong et al. 2014). In this study, however, the mean mechan-
significant correlations were identified (Spearman correlation coefficient, \( r \)). No difference was found between the male and female rats, which is consistent with mechanical sensitization induced by NGF injection site (X) and the subsequent NMDA injection site (c). The interpretation of these results is not equal to 0.05).

Whereas our results suggest that NGF induces muscle mechanical sensitization via increasing the expression of NMDA receptors in the peripheral endings of masseter mechanoreceptors, other studies suggest that presynaptic NMDA receptors on the central terminals of primary afferents may also induce hyperexcitability during injury via facilitation of the release of glutamate and substance P (SP) in the dorsal horn (Liu et al. 1997; Yan et al. 2013). However, conflicting results have been observed where intrathecal NMDA did not evoke SP release in one in vivo study (Navarian et al. 2008) and NMDA was found to decrease EPSPs recorded in spinal cord slices in another (Bardoni et al. 2004). The interpretation of these results is not comparable with glutamate-induced mechanical sensitization in slow A\(\delta\)-fibers than fast A\(\delta\)-fibers in the female rats but not male rats. This result suggests that NGF may have a different sensitizing effect on putative mechanonociceptors in the female rats compared with the male rats. This effect was not related to estrogen as no correlation was observed between plasma estrogen concentration and the level of sensitization, although previously it has been found that estrogen may increase masseter muscle sensitivity to NMDA by increasing peripheral NMDA receptor expression (Dong et al. 2007; McRoberts et al. 2007). Sex-related differences in masseter muscle nociception have been documented previously in human and animal experiments. For example, healthy women exhibited greater sensitization following NGF injection into the masseter muscle than healthy men (Svensson et al. 2003, 2008a). A higher dose of ketamine, an NMDA receptor antagonist, was required to attenuate glutamate-evoked masseter pain in women than men, suggesting a similar sex-related difference in peripheral NMDA receptor expression (Cairns et al. 2006; Castrillon et al. 2012). We previously found that NGF induced a greater mechanical sensitization in female rats than in male rats, which was associated with a greater increase in NMDA receptor expression by masseter ganglionic neurons in the female rats (Wong et al. 2014). These results suggest there is a marked difference in the effect of NGF on peripheral NMDA receptor expression between the sexes.

In this study, a sex-related difference was observed in the level of NMDA-evoked mechanical sensitization following NGF injection, with the male rats exhibiting a greater decrease in afferent mechanical threshold than the females. Further examination found that NGF induced a greater NMDA-induced mechanical sensitization in the slow A\(\delta\)-fibers than fast A\(\delta\)-fibers in the female rats but not male rats. This result suggests that NGF may have a different sensitizing effect on putative mechanonociceptors in the female rats compared with the male rats. This effect was not related to estrogen as no correlation was observed between plasma estrogen concentration and the level of sensitization, although previously it has been found that estrogen may increase masseter muscle sensitivity to NMDA by increasing peripheral NMDA receptor expression (Dong et al. 2007; McRoberts et al. 2007). Sex-related differences in masseter muscle nociception have been documented previously in human and animal experiments. For example, healthy women exhibited greater sensitization following NGF injection into the masseter muscle than healthy men (Svensson et al. 2003, 2008a). A higher dose of ketamine, an NMDA receptor antagonist, was required to attenuate glutamate-evoked masseter pain in women than men, suggesting a similar sex-related difference in peripheral NMDA receptor expression (Cairns et al. 2006; Castrillon et al. 2012). We previously found that NGF induced a greater mechanical sensitization in female rats than in male rats, which was associated with a greater increase in NMDA receptor expression by masseter ganglionic neurons in the female rats (Wong et al. 2014). These results suggest there is a marked difference in the effect of NGF on peripheral NMDA receptor expression between the sexes.

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straightforward as activation of presynaptic glutamate receptors may shunt the action potential propagation and reduce neurotransmitter release, a mechanism similar to the proposed role of GABA<sub>A</sub> receptors in primary afferent depolarization (Bardoni 2013). Further experiments are needed to determine what the consequence of increasing central presynaptic NMDA receptors would be on synaptic transmission.

It is also likely that sex-related differences in NGF-induced mechanical sensitization of the masseter muscle result from a mechanism other than increased peripheral NMDA receptor expression. For example, we previously reported that intramuscular injection of NGF increased the expression of the neuropeptides SP and calcitonin gene-related peptide (CGRP) by masseter ganglion neurons that coexpressed NMDA receptors but only in female rats (Wong et al. 2014). However, peripheral NMDA receptor-induced mechanical sensitization of masticatory muscle afferent fibers in female rats is not inhibited by local injections of antagonists for SP or CGRP receptors (Gazerani et al. 2010). The greater expression of neuropeptides by masseter muscle afferent fibers in female rats after NGF treatment could result in an increased central release of SP/CGRP, which may increase the excitability of neurons in the trigeminal subnucleus caudalis (Coste et al. 2008; Meng et al. 2009). This central mechanism may contribute more to NGF-induced mechanical sensitization in female rats than in male rats (Latremoliere and Woolf 2009). Thus a combination of peripheral and central sensitization may explain the observed sex-related difference in the effect of intramuscularly injected NGF on masseter muscle mechanical threshold.

In this study, we found NGF increases the mechanical sensitivity of masseter muscle nociceptors to NMDA receptor activation. These results suggest that NGF, an important mediator of pain, may induce mechanical allodynia, in part, by increasing the expression of NMDA receptors in the peripheral endings of muscle nociceptors. In an earlier study, NGF increased the median soma size of masseter ganglion neurons expressing NMDA receptors, which suggests that peripheral NMDA receptors may be part of the phenotypic change that occurs in muscle nociceptors during injury and inflammation (Wong et al. 2014). These results may be relevant to humans, as NMDA receptors have been found in human masseter muscle afferent fibers (Wong et al. 2014). Our results merit further study into the role of peripheral NMDA receptors in craniofacial muscle pain development.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
H.W. and B.E.C. conception and design of research; H.W. and X.-D.D. performed experiments; H.W. analyzed data; H.W. interpreted results of experiments; H.W. prepared figures; H.W. drafted manuscript; H.W., X.-D.D., and B.E.C. edited and revised manuscript; B.E.C. approved final version of manuscript.

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