Intramuscular Temperature Modulates Glutamate-Evoked Masseter Muscle Pain Intensity in Humans

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Aims: To determine whether glutamate-evoked jaw muscle pain is altered by the temperature of the solution injected. **Methods:** Sixteen healthy volunteers participated and received injections of hot (48°C), neutral (36°C), or cold (3°C) solutions (0.5 mL) of glutamate or isotonic saline into the masseter muscle. Pain intensity was assessed with an electronic visual analog scale (eVAS). Numeric rating scale (NRS) scores of unpleasantness and temperature perception, pain-drawing areas, and pressure pain thresholds (PPTs) were also measured. Participants filled out the McGill Pain Questionnaire (MPQ). Two-way or three-way repeated measures ANOVA were used for data analyses. **Results:** Injection of hot glutamate and cold glutamate solutions significantly increased and decreased, respectively, the peak pain intensity compared with injection of neutral glutamate solution. The duration of glutamate-evoked pain was significantly longer when hot glutamate was injected than when cold glutamate was injected. No significant effect of temperature on pain intensity was observed when isotonic saline was injected. No effect of solution temperature was detected on unpleasantness, heat perception, cold perception, area of pain drawings, or PPTs. There was a significantly greater use of the "numb" term in the MPQ to describe the injection of cold solutions compared to the injection of both neutral and hot solutions. **Conclusion:** Glutamate-evoked jaw muscle pain was significantly altered by the temperature of the injection solution. Although temperature perception in the jaw muscle is poor, pain intensity is increased when the muscle tissue temperature is elevated. *J Oral Facial Pain Headache 2015;29:158–167. doi: 10.11607/ofph.1332*

Key words: *glutamate-evoked pain, musculoskeletal pain, pain assessment, temperature measurement*

Chronic muscle pain conditions are reported by more patients who seek medical attention compared to any other form of pain.[1,2] Of these muscle pain conditions, jaw muscle pain associated with temporomandibular disorders (TMD) is a common complaint and affects about 10% to 15% of the population.[3–6] Localized myalgia and tenderness upon manual palpation of the jaw muscles, which are evident in myofascial TMD, suggest that this pain could be due to altered pain sensitivity in the muscle tissue. However, the pathophysiologic mechanisms that result in chronic jaw muscle pain conditions are still incompletely understood. Animal research has suggested that activation of peripheral N-methyl-D-aspartate (NMDA) receptors, which are expressed by jaw muscle nociceptors, may contribute to pain and sensitivity in myofascial TMD.[7–9] In healthy humans, injections of glutamate (0.1 to 1 M) into the masseter muscle produce pain through activation of peripheral NMDA receptors, and this pain resembles some of the aspects of the muscle pain in myofascial TMD patients.[10–15] This has led to the use of glutamate injections into the jaw muscles as a model of myofascial TMD pain.[13]

Animal research indicates that leg muscle nociceptors respond to noxious heat,[16] but it is unknown how temperature affects jaw muscle nociceptors. Several lines of evidence indicate that the transient receptor potential vanilloid subfamily member 1 (TRPV1) channel, which responds to noxious heat (> 42°C), protons, and capsaicin, is found

in the masseter muscle.[17–21] Further, NMDA receptors and TRPV1 channels functionally interact via Ca2+ calmodulin-dependent protein kinase II (CaMKII) and protein kinase C (PKC) signaling cascades to contribute to mechanical hyperalgesia.[17,18,20] In healthy humans, intramuscular injection of glutamate causes a sensitization to subsequent administration of capsaicin, whereas capsaicin is associated with a desensitization to subsequent injection of glutamate.[22] Since TRPV1 channels can also be activated by noxious heat, there is the potential for an interaction between solution temperature and glutamate-evoked jaw muscle pain.

Although mild muscle pain can be evoked by high-temperature isotonic saline in humans,[23] there are no data on whether muscle pain evoked by glutamate is altered by the temperature of the solution injected. These observations raised the possibility that there may be a functional interaction between muscle temperature and pain perception and to the hypothesis that elevated muscle temperature increases glutamate-evoked jaw muscle pain in human participants. To test this hypothesis, the present study was conducted to determine whether glutamate-evoked jaw muscle pain is altered by the temperature of the solution injected.

Materials and Methods

Participants

A total of 16 healthy volunteers were recruited from Aarhus University (4 males, 12 females; mean age [± SEM] 26.5 years ± 1.2) (range 20 to 35 years). The participants had no signs or symptoms of TMD according to the Research Diagnostic Criteria for TMD (RDC/TMD)[24] and no complaints of orofacial pain. The study protocol was approved by The Central Denmark Region Committees on Biomedical Research Ethics (No.1-10-72-75-13) and followed the guidelines of the World Medical Association Declaration of Helsinki, with thorough written and oral information about the experiment provided to the participants before they signed the informed consent document. Exclusion criteria included pregnancy (participant-based report), presence of a score greater than 8 on the Beck Depression Inventory-Second Edition (BDI-2), fibromyalgia,[25] and the use of analgesic(s) within 2 days of the planned experimental sessions.

Study Design

The protocol and sequence followed are illustrated in Fig 1. Glutamate or isotonic saline, in a paired-sequence and randomized order, was injected into the right or left masseter muscle. Two injections were given in a double-blinded manner 20 minutes apart in each session. Participants attended a total of three sessions with a minimum interval of 3 days between sessions. All assessments were made by the same experimenter (HS). Intramuscular injections of sterile solutions containing monosodium glutamate (0.5 M, 0.5 mL) (Ajinomoto Co) and isotonic saline (0.5 mL) were injected into the masseter muscle over 10 seconds with a 27-G hypodermic needle attached to a disposable syringe and followed methods already described.[10–14,22,26–34] The temperatures of the solutions were adjusted to either cold (3˚C), neutral (36˚C), or hot (48˚C) prior to the injection. Neutral and hot solutions were produced by immersing the solution in a thermostatically regulated water bath set to 42˚C and 58˚C, respectively. The water baths were set at temperatures higher than the target temperatures to compensate for the temperature loss due to subsequent handling (eg, drawing up into the syringe, etc).[23] Moreover, in order to assume the temperature of the solution at the time of injection, the natural course of temperature of solutions (room temperature: 25˚C) was recorded with a flexible temperature probe (0.8 mm, Ellab A/S) connected to a thermometer (DM852, Ellab A/S). The cold solution was produced by immersion of the solution in ice to obtain the 3˚C target temperature. The temperature-adjusted glutamate solutions were injected into the most prominent point of the masseter muscle, which prior to injection was identified during tooth clenching; the contralateral muscle was injected with temperature-adjusted isotonic saline. Participants were given careful instructions to keep relaxing the jaw while solutions were injected into the muscle. The injection order was randomized between the three sessions by a clinical assistant. The participants were unaware of the temperature or content of the injections. Therefore, participants were randomly assigned to receive six intramuscular injections of the following combinations: *(1)* cold saline–cold glutamate, *(2)* neutral saline–neutral glutamate, and *(3)* hot saline–hot glutamate.

Assessments of Pain and Pain-Related Responses

The participants continuously scored their pain intensity on an electronic visual analog scale (eVAS), with the lower extreme marked with “no pain” and the upper extreme with “most pain imaginable.” Two separate recordings of 15 minutes each were performed per session; the recordings started at the time of an injection (Fig 1). Three parameters from the output of the eVAS were considered: the area under the curve (eVAS AUC), the duration of the pain (eVAS duration), and the peak pain value (eVAS peak). The eVAS AUC was calculated by summation of all of the eVAS values for each 15-minute period.

Pain-related responses consisted of area of pain drawing, unpleasantness, and temperature perception (Fig 1). The participants were asked to draw, from a lateral view of the face, the distribution of the perceived pain level that they felt immediately after an injection. The pain drawings were digitized (Sigma Scan Pro 4.01.003) and their area expressed in arbitrary units ([au]: 1 mm^2).[10] The level of unpleasantness that they felt immediately after an injection was scored on a numeric rating scale (NRS) ranging from 0 ("not unpleasant") to 10 ("worst unpleasantness imaginable"). Heat and cold perceptions that they felt immediately after an injection were scored on a NRS ranging from 0 ("neutral") to 10 ("painful heat" or "painful cold"). These responses were determined at 5 minutes after an injection (during peak pain) (Fig 1).

Assessment of Pressure Pain Threshold

An electronic pressure algometer (Somedic) was used to measure the pressure pain threshold (PPT). The PPT is defined as the amount of pressure (kPa) that the participant first perceives to be painful. The algometer probe (1-cm^2 area) was applied perpendicularly to the masseter at the site of injection as well as to the nail of the dominant index finger. During the pressure stimulation, the participants were instructed to keep their teeth slightly apart (without intercuspal contacts) with minimum voluntary contraction and to focus their attention on the experimental task. The participants pushed a button to stop the pressure stimulation when pain was felt. The PPTs were determined in triplicate at baseline (ie, before any injection) and at 5 and at 15 minutes after an injection (Fig 1). The pressure was delivered with a constant application rate of 30 kPa/s.

Assessment of Psychophysical Quality of Pain

Each participant completed a Danish or an English version of the McGill Pain Questionnaire (MPQ)[35,36] to obtain a qualitative description of the pain sensations induced. The assessments were performed immediately after an injection (Fig 1).

Measurement of Changes in Solution, Skin Surface, and Intramuscular Temperature

The change in temperature of hot, neutral, and cold solutions over time when they were placed in a syringe at room temperature was recorded with a flexible temperature probe (0.8 mm, Ellab A/S) connected to a thermometer (DM852, Ellab A/S). In a subgroup of participants (n = 5), the skin surface temperature was recorded using a compact infrared thermal-imaging camera (FLIR E60bx, FLIR Systems Inc). The intramuscular temperature was also recorded with a flexible temperature probe (0.8 mm, Ellab A/S) connected to a thermometer (DM852, Ellab A/S) which also previously has been used to perform intramuscular microdialysis.[37] To insert the intramuscular temperature probe, the skin surface was first anesthetized with EMLA cream (AstraZeneca AB) for 30 minutes. A sterile acrylic 6-mm-thick plastic plate (10 × 40 mm) was then placed over the masseter muscles (Fig 2). This plate had two 1.3-mm-wide guide holes drilled in it at a distance of 10 mm apart; one at a 90-degree angle to the surface and the other at a 45-degree angle. To insert the probe, a standard catheter (18G, Venflon, Becton Dickinson Infusion Therapy AB) was inserted into the masseter muscle through the 45-degree guide hole and the needle then removed, leaving the end of the plastic just inside the muscle tissue (approximately 10 to 12 mm from the skin surface). A solution of isotonic saline at one of the three temperatures was injected via the perpendicular guide hole (90-degree angle to the surface) of the acrylic plate into the masseter muscle. The plastic plate was constructed so that when both the probe and needle were inserted, the intramuscular temperature probe was approximately 5 mm from the tip of the injection needle (Fig 2). Participants attended a total of three sessions with a minimum interval of 3 days between sessions.

Statistical Analyses

SPSS 20 (IBM) was utilized for statistical analyses. Two-way repeated measures analysis of variance (ANOVA) was used to test differences in eVAS peak pain, eVAS pain duration, eVAS AUC, area of pain drawing, and MPQ score with the repeated measurement factors of solution (saline and glutamate) and temperature (cold, neutral, and hot). Power analysis based on a two-way repeated measures ANOVA indicated that with a minimum of nine subjects per group, a 25% difference could be detected with a risk of type I and type II errors of 5%, respectively. However, because of the unknown effect size, a larger number of subjects were included. Ranked two-way repeated measures ANOVA was used to test differences in unpleasantness level and temperature perception with the repeated measurement factors of solution (saline and glutamate) and temperature (cold, neutral, and hot). Three-way repeated measures ANOVA was used to test differences in the PPT with repeated measurement factors of solution (saline and glutamate), temperature (cold, neutral, and hot), and time (baseline, 5, and 15 minutes after an injection). The Tukey Honestly Significant Difference (Tukey HSD) test with corrections for multiple comparisons was used for post-hoc analyses. The difference in the words chosen from the MPQ under the different experimental conditions was analyzed with the use of Cochran *Q* test. All results are presented as means ± SEM. Values of $P < .05$ were considered statistically significant.

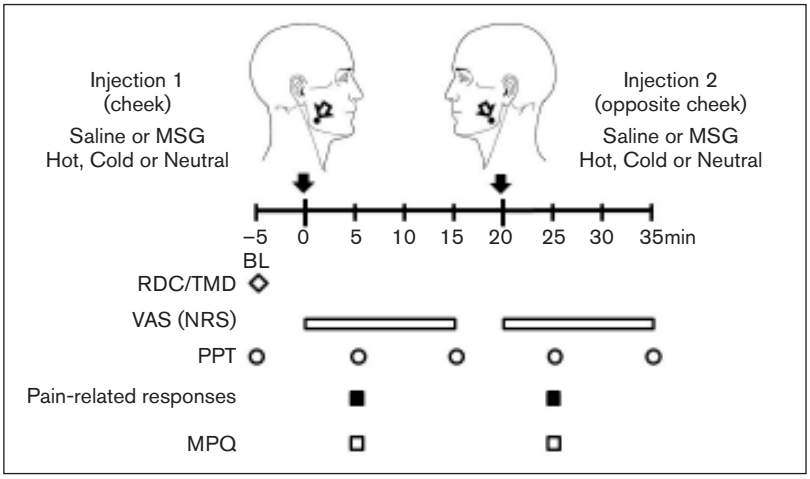


Fig 1 Schematic illustration of the experimental protocol. Participants received a total of six injections bilaterally into masseter muscles; two injections were made per session. The site of injection was randomized between the left and right masseter muscle. The top diagram shows the six possible injection combinations. The participants continuously scored their pain intensity on an eVAS. Two separate recordings of 15 minutes each were performed per session; the recordings started at the time of an injection. The PPTs were determined at baseline (ie, before any injection) and at 5 minutes and 15 minutes after an injection. Pain-related responses (pain drawing area, unpleasantness, and temperature perception) and MPQ were determined at 5 minutes after an injection (during peak pain). BL = baseline; MSG = monosodium glutamate solution.

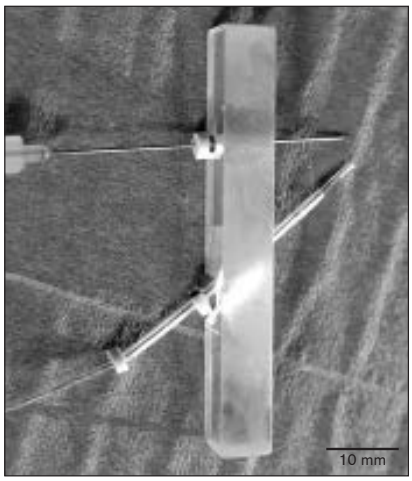


Fig 2 Photograph showing the plastic plate used to guide the insertion of the intramuscular temperature probe and injection needle. The plastic plate was attached with surgical tape to the skin overlying the masseter muscle. The intramuscular temperature probe was inserted into the muscle at a 45-degree angle to the skin surface, while the injection needle was inserted perpendicular to the skin surface. In this manner, the intramuscular temperature probe was localized at a distance of 5 mm from the tip of the injection needle. The filled bar indicates 10 mm.

Results

Pain and Pain-Related Responses

Injection of glutamate produced significantly more pain than injection of isotonic saline, regardless of solution temperature. The solution temperature had a significant effect on the magnitude of eVAS peak pain (Fig 3a) and duration of pain (Fig 3b) evoked by injection of glutamate (ANOVA: $df = 2$; $F > 4.933$, $P < .023$), but did not affect the magnitude of pain evoked by isotonic saline ($df = 2$; $F < 0.961$, $P > .394$). For glutamate injections, post-hoc comparisons showed eVAS peak pain was significantly greater after injection of hot solutions than both neutral or cold solutions, and that cold solutions produced significantly less pain than neutral solutions (Tukey: $P < .050$). The duration of pain after injection of hot glutamate solutions was significantly longer than after injection of cold glutamate solutions (Tukey: $P = .022$). There was no significant effect of solution temperature on the eVAS AUC (Fig 3c) ($df = 2$; $F = 3.721$, $P = .051$).

Areas of pain drawings after injection of glutamate were significantly larger than those after injection of isotonic saline ($df = 1$; $F = 27.943$, $P < .001$). There was no significant effect of temperature on the area of pain for either solution (Table 1) ($df = 2$; $F = 2.465$, $P = .121$). No significant difference was seen in the NRS scores for unpleasantness, heat perception, and cold perception over the sessions, which indicates that the participants were unable to differentiate hot from cold injections (Table 1) ($df = 2$; $F < 1.000$, $P > .333$).

Pressure Sensitivity

There were no significant differences in the PPTs of the masseter muscle (Fig 4a) ($df = 4$; $F = .634$, $P = .648$) or the hand (Fig 4b) ($df = 4$; $F = .519$, $P = .724$) when glutamate and isotonic saline injections into the masseter muscle were compared. There was no effect of injection solution temperature on PPT.

Quality of Pain

Temperature had a significant effect on the miscellaneous word scores chosen from the MPQ (Table 2) ($df = 2$, $F = 5.416$, $P = .018$). Although post-hoc comparisons of the scores of miscellaneous words revealed no significant difference between

Table 1 Comparison of Pain-Related Responses Evoked by Injections of Temperature-Controlled Glutamate and Isotonic Saline in 16 Participants

	Glutamate			Saline		
	Cold	Neutral	Hot	Cold	Neutral	Hot
Pain area (mm^2)	69.9 (19.0)	99.6 (19.6)	90.0 (15.9)	21.2 (9.6)	11.0 (3.9)	9.1 (2.1)
Unpleasantness (0–10)	5.0 (0.5)	5.1 (1.9)	5.3 (0.6)	2.5 (0.4)	1.9 (0.4)	1.8 (0.3)
Heat perception (0–10)	0.0 (0.0)	0.4 (0.26)	0.0 (0.0)	0.0 (0.0)	0.1 (0.1)	0.0 (0.0)
Cold perception (0–10)	0.2 (0.2)	0.4 (0.44)	0.0 (0.0)	0.1 (0.1)	0.0 (0.0)	0.0 (0.0)

Mean values (± SEM) with F and *P* values for the two-way measured repeated ANOVA and the ranked two-way measured repeated ANOVA.

Table 2 Comparison of MPQ Scores Evoked by Injections of Temperature-Controlled Glutamate and Isotonic Saline in 16 Participants

	Glutamate			Saline		
	Cold	Neutral	Hot	Cold	Neutral	Hot
MPQ sensory (0–42)	9.3 (1.9)	10.5 (1.8)	11.2 (1.7)	2.2 (0.7)	2.2 (0.6)	1.3 (0.3)
MPQ affective (0–13)	0.5 (0.3)	0.6 (0.3)	0.5 (0.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
MPQ evaluative (0–5)	0.8 (0.4)	0.7 (0.3)	0.9 (0.4)	0.0 (0.0)	0.1 (0.1)	0.1 (0.1)
MPQ miscellaneous (0–17)	3.3 (0.7)**	1.4 (0.4)	1.3 (0.4)	0.8 (0.3)	0.3 (0.1)	0.4 (0.2)
MPQ (PRI) total (0–77)	13.8 (2.8)	13.0 (2.2)	13.8 (2.1)	2.9 (0.9)	2.4 (0.6)	1.8 (0.4)

Mean values (± SEM) with F and *P* values for the two-way measured repeated ANOVA. The post-hoc comparisons are performed with Tukey's test.
* Indicates significant difference between the two solutions with changing temperature (two-way measured repeated ANOVA).
** Indicates significant difference compared to any other temperature of glutamate (Tukey test).

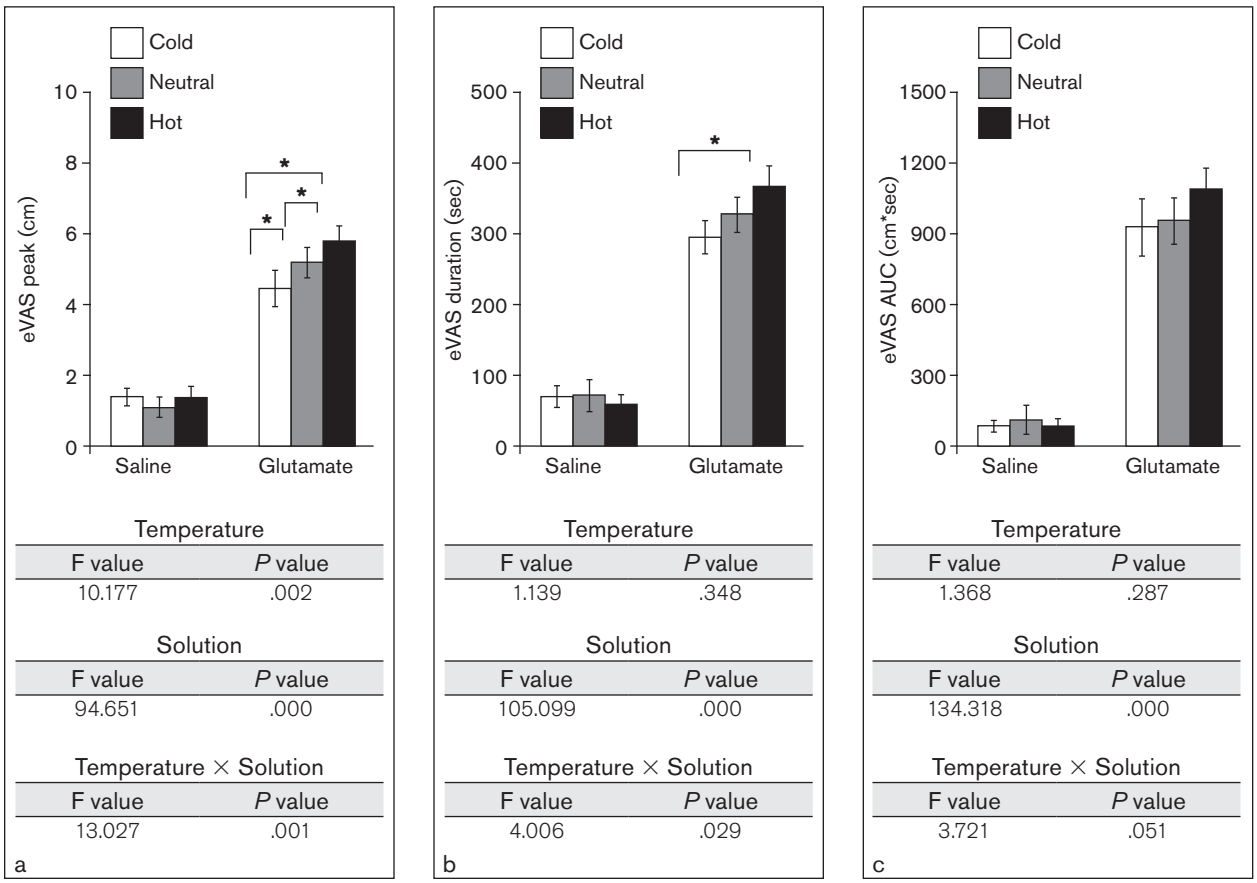
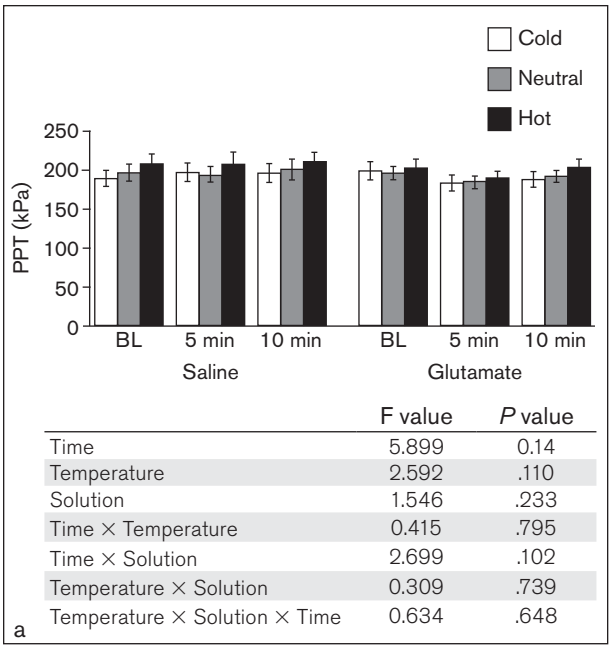


Fig 3 Bar graphs illustrate the averages for the various eVAS parameters in response to the intramuscular injection of isotonic saline and glutamate at various temperatures. They show the mean (± SEM, n = 16) of peak pain score (**a**: eVAS peak) and pain duration (**b**: eVAS duration), and are under the curve (**c**: eVAS AUC) after injections of each solution (0.5 mL) with three target temperatures of cold, neutral, and hot. * Indicates significant difference between the two injections (Tukey tests; $P < .050$) (n = 16).

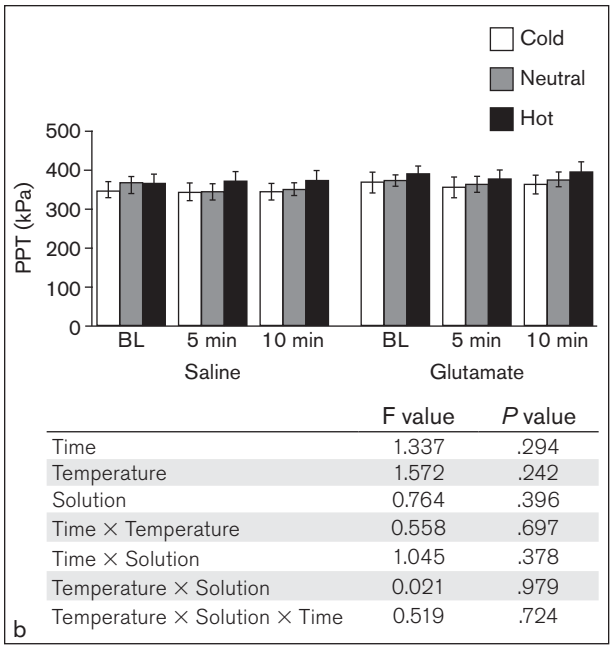
Temperature		Solution		Temperature × Solution	
F value	*P* value	F value	*P* value	F value	*P* value
0.425	.662	27.943	.000	2.465	.121
0.465	.637	0.368	.553	0.164	.850
1.000	.393	1.000	.333	1.000	.333
3.457	.083	0.261	.227	0.261	.617

Temperature		Solution		Temperature × Solution	
F value	*P* value	F value	*P* value	F value	*P* value
0.455	.643	37.146	.000	1.391	.281
0.080	.923	7.567	.015	0.080	.923
0.093	.912	7.642	.014	0.193	.827
6.655	.009	10.565	.005	5.416	.018*
0.082	.921	33.826	.000	0.227	.800



	F value	*P* value
Time	5.899	0.14
Temperature	2.592	.110
Solution	1.546	.233
Time × Temperature	0.415	.795
Time × Solution	2.699	.102
Temperature × Solution	0.309	.739
Temperature × Solution × Time	0.634	.648

a

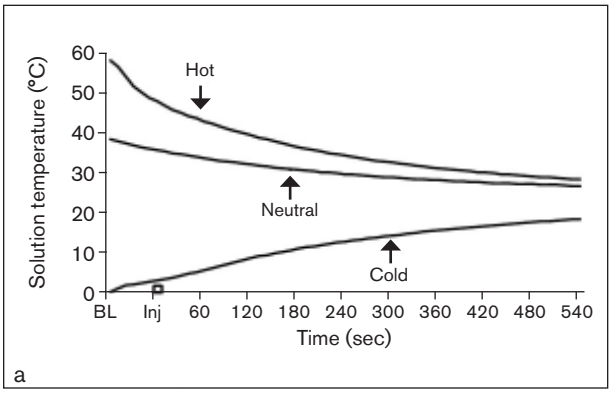


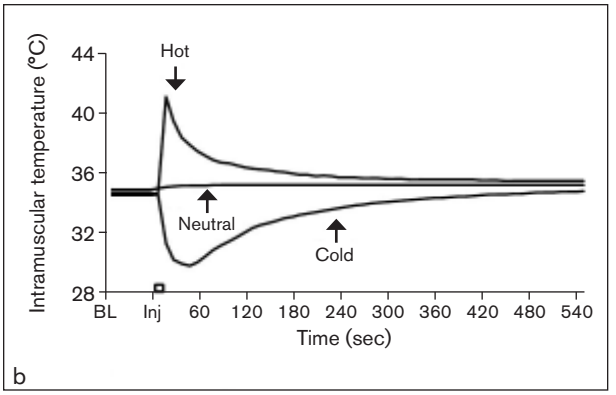
	F value	*P* value
Time	1.337	.294
Temperature	1.572	.242
Solution	0.764	.396
Time × Temperature	0.558	.697
Time × Solution	1.045	.378
Temperature × Solution	0.021	.979
Temperature × Solution × Time	0.519	.724

b

Fig 4 Bar graphs show the average PPT at baseline (ie, before any injection), at 5 and at 15 minutes after an intramuscular injection of saline and glutamate into the masseter muscle or hand. They show the mean (± SEM, n = 16) PPT in the **(a)** masseter muscle and **(b)** dominant hand after injections of each solution (0.5 mL) with three target temperatures of cold, neutral, and hot. There were no significant differences in the PPTs of the masseter muscle or the hand when the effects of glutamate and isotonic saline injections into the masseter muscle or hand were compared. BL = baseline.

each temperature for isotonic saline injections (Tukey: $P > .102$), they did identify significant differences in the word scores for the glutamate injection between cold and neutral and neutral and hot solutions, respectively (Tukey: $P < .004$). There were no other significant differences in MPQ scores (Table 2) ($df = 2$, $F < 1.391$, $P > .281$). The word most chosen by the participants to describe their cold glutamate solution–evoked pain in the MPQ was "numb" (Cochran *Q* test; $P < .006$).





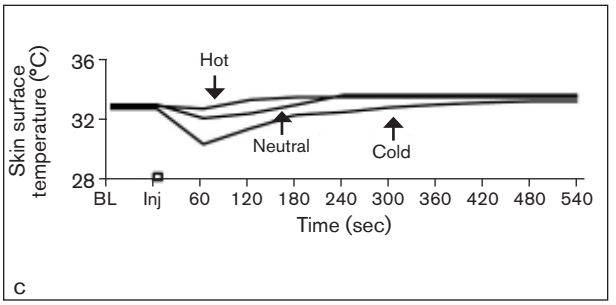


Fig 5 Mean (n = 5) change in temperature of hot, neutral, and cold isotonic saline solutions over time when placed in a room at **(a)** room temperature (20°C), as well as **(b)** intramuscular, and **(c)** skin surface temperature after solution injection into the masseter muscle. The white bar above the horizontal axis indicates the time of injection. The hot solutions were ~ 48°C at the time of injection and raised the intramuscular temperature to 41.1 ± 0.5°C, while the cold solutions were ~ 3 °C at the time of injection and reduced the intramuscular temperature to 29.9 ± 0.6°C. BL = baseline; Inj = injection.

Change in Tissue Temperature

The hot and cold solutions of isotonic saline were ~ 48˚C and ~ 3˚C, respectively, at the time of their injection into the masseter muscle (Fig 5a). Hot solutions raised the intramuscular temperature from 34.6 ± 0.5˚C to 41.1 ± 0.5˚C in 10 seconds, and cold solutions reduced the intramuscular temperature from 34.7 ± 0.5˚C to 29.9 ± 0.6˚C in 30 seconds (Fig 5b). Peak temperature changes produced by the injections lasted for less than 10 seconds (hot) to as long as 60 seconds (cold), after which time there was a rapid return to the baseline muscle temperature. The skin surface temperature was decreased immediately after the injection of solutions regardless of the temperature of the solution, but the decrease by cold solutions was the largest (Fig 5c).

Discussion

The main finding of this study was that the temperature of the injected glutamate solution significantly affected the pain intensity rating. In particular, intramuscular injection of hot (48˚C) glutamate into the masseter muscle induced more muscle pain than injection of neutral (36˚C) glutamate, while injection of cold (3˚C) glutamate significantly reduced pain compared with neutral glutamate injections. Despite finding that the solution temperature affected pain ratings after glutamate injections into the masseter muscle, there was no evidence that participants could perceive the temperature of the injected solutions as either cold or hot. Further, neither hot nor cold isotonic saline solutions evoked pain that was different from neutral solutions. These findings suggest that large temperature changes within the masseter muscle are able to modulate pain sensitivity to painful chemical stimulation, but that stimulation of the muscle by thermal stimuli that would be painful if applied to the skin is not perceived by healthy participants. Thus, consistent with previous findings, these results indicate that humans may not be able to perceive changes in muscle temperature as either hot or cold.[23]

It is challenging to explain why elevated intramuscular temperatures enhance glutamate-evoked pain without causing participants to report any perception of heat. It is known that masseter muscle nociceptors express functional TRPV1 channels, and that capsaicin injections into the masseter muscle of human participants are painful.[22,38,39] Although injection of capsaicin into the masseter muscle induces intense pain, participants do not choose words on the MPQ to suggest that the pain is perceived as heat pain.[22] This suggests that activation of TRPV1 channels in skeletal muscle, unlike skin or oral mucosa, is not perceived as a burning-like pain and that the failure to perceive heat stimulation may not be due to a lack of activation of afferent fibers in the muscle by the heat stimulus, but rather to the central processing of these signals. It might be expected that the elevated temperatures produced by the hot solutions injected in this study would activate TRPV1 and/or other thermoreceptors on nociceptors and induce the release of

neuropeptides such as substance P and bradykinin (BK). Substance P is thought to contribute to heat hyperalgesia associated with inflammation or nerve injury,[40,41] and many lines of evidence indicate that bradykinin can evoke thermal hyperalgesia by activating a neuronal membrane ionic current[42] in rodents,[43] nonhuman primates,[44] and humans.[45] Moreover, the sensitizing effect of BK is dependent on tissue temperature: it is clearly observed above 35˚C but not at lower temperatures in the human arm.[46] Therefore, it can be postulated that injections of hot solutions cause the release of neuropeptides, which sensitize masseter muscle nociceptors to increase glutamate-evoked masseter muscle pain.

A consistent finding in the present study was the difference in the miscellaneous score of MPQ between the cold and neutral glutamate injections. Although previous research has shown that the MPQ scores are not significantly different between glutamate-evoked jaw muscle pain in healthy participants and patients with persistent myofascial TMD pain,[13] the miscellaneous score of cold glutamate-evoked pain was higher than that of neutral glutamate-evoked pain in the present study. This was due to the high frequency of use of the term "numb" to describe the pain produced by the injection of the cold glutamate solution. This suggests that the participants, although not able to perceive these injections as cold, were nonetheless aware of a decrease in sensation from the muscle. Measurement of intramuscular temperature showed that the cold solution (3˚C) reduced intramuscular temperature to less than 30˚C for about one minute at a distance of 5 mm from the needle tip. Research has shown that Na/K pump activity decreases with temperature reduction,[47–49] and that cooling leads to slowed nerve conduction velocity,[50] which likely explains the perceived loss of sensation from the muscle. In addition, cold solution injections maintained the intramuscular temperature at less than 30˚C for 1 minute after the injection, which likely prevented sensitization induced by the release of BK.[46] With regard to the inability of the participants to perceive the cold injections as cold, it is possible that muscle nociceptors do not have cold receptors. However, to the authors' knowledge, the expression by afferent fibers that innervate the masseter muscle of moderate threshold cold-sensitive receptors, including the TRP cation channel subfamily M member 8 (TRPM8) channel, has not been investigated. Therefore, slowed nerve conduction coupled with a lack of temperature-sensitive receptors may have contributed to the decrease in glutamate-evoked pain intensity and numbness reported by the participants after cold glutamate injections. Even if TRPM8 is expressed by masseter muscle afferent fibers, activation of the receptor may not be perceived as cold. For example, the application of cold stimuli to certain internal organs that do express TRPM8 (prostate,[51] colon,[52] etc) is not reported to evoke sensations of cold.

In the present study, the area of pain after injection of glutamate was found to be greater than after injection of isotonic saline; however, there was no effect of the temperature of the injected solution on the area of pain drawing.[10,12,22] In general, changes in pain area are less sensitive to alterations in pain intensity than other measures. In the present study, it was suggested that the amount of change in the pain scores was insufficient to alter the perceived area of pain.

The strengths and limitations of this study should be noted. Its strengths include the design, as all injections were performed in a double-blind manner, thereby minimizing the chance of bias, and also the incorporation of measurements of intramuscular temperature. However, one limitation of the technique to measure intramuscular temperature in this study was that it was measured at a point 5 mm from the tip of the injection needle, which suggests that the reported temperatures likely underestimated the actual temperatures achieved at the needle tip. In an earlier study, Graven-Nielsen et al reported that intramuscular temperatures produced from the injection of hot (49˚C) and cold (5˚C) solutions were essentially the same as the temperature of the injected solution; however, in their study, the temperature probe was incorporated into the injection catheter and a much larger volume of solution (1.5 mL) was injected.[23] A second limitation was that the injection was performed 30 minutes after the anesthetization to prevent cutaneous sensory input from the skin overlying the masseter muscle in the additional experiment of the measurement of changes in tissue temperature. When the injections were performed it was observed that the temperature of the skin returned to the temperature that existed before the anesthetization. Nevertheless, it was noted that the skin surface temperature was temporarily decreased immediately after the injection of solution regardless of the temperature of the injected solution. Since skin surface temperature consistently decreased under all experimental conditions, it is unlikely that changes in skin sensitivity played a significant role in influencing areas of pain drawings or PPT values obtained in the present study. A third possible limitation of the present study was that muscle pain was assessed only in young participants. It has been reported that aged animals have more severe muscle fiber damage[53] and a slower recovery from muscle damage than younger animals.[54] Taguchi et al demonstrated that the mechanical hyperalgesia in the deep tissue after exercise lasted longer in aged (130-week-old) rats than in young (7-week-old) rats.[55] In contrast, current

evidence suggests that older women (average age 67.4 years) compared with younger women (average age 23.6 years) experience a similar magnitude and duration of pain after exercise.[56] Future studies testing a larger age span may be warranted in order to investigate this discrepancy between age-related differences in animal and human studies. Moreover, the NMDA receptor antagonist ketamine, which has been shown to reduce glutamate-induced masseter muscle pain and mechanical sensitization in healthy men,[7] is ineffective at the same dose when used against glutamate-evoked pain mechanical sensitivity in healthy women[14] or against myofascial pain in predominantly female patients with persistent myofascial TMD pain.[29] Thus, future studies testing a larger sample size including males and females are needed in order to investigate the differences between men and women in temperature-adjusted glutamate-evoked pain.

Conclusions

Glutamate-evoked jaw muscle pain was altered by the temperature of the injected solution. Although temperature perception in the jaw muscle is poor, pain intensity is increased when the muscle tissue temperature is elevated. These features suggested that large temperature changes in the muscle can modulate muscle pain without any perception of temperature in healthy participants. These findings indicate that pain sensitivity in the human jaw muscle can be modulated by altering intramuscular temperature.

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