Characteristics of Glutamate-Evoked Pain in the Masseter Region: Differences Between Targeted Injections in Subcutaneous, Muscle, and Bone Tissues

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Aims: To investigate whether glutamate-evoked pain in the masseter region differs between three different depths of injection, targeting subcutaneous, muscle, and bone tissues. Methods: A total of 16 healthy volunteers participated and, in a randomized order, received injections of glutamate (1.0 M, 0.2 mL) and isotonic saline (0.9%, 0.2 mL) in the masseter region that targeted subcutaneous, intramuscular, and bone surface tissues. Following injection, pain intensity was measured using electronic visual analog scale (eVAS) and numeric rating scale (NRS) scores of unpleasantness, tiredness, tension, soreness, and stiffness. Pressure pain sensitivity (PPS), pain drawing areas, and McGill Pain Questionnaire (MPQ) scores were also assessed. Repeated-measures analysis of variance, McNemar test, and Tukey post hoc tests were used for statistical analyses. P < .05 was considered statistically significant. Results: Overall, subcutaneous injections induced significantly more unpleasantness and pain than intramuscular injections, and PPS scores evoked after glutamate injection at the surface of the bone were significantly higher than after intramuscular glutamate injection. Subcutaneous glutamate injections were more often described as “sharp” and “pinching.” Conclusion: The subcutaneous injection was more painful and unpleasant than the intramuscular injection. The glutamate injection at the surface of the bone sensitized the deep pain tissues to pressure stimulation. Clinically, it may be difficult to differentiate between the source or site of pain originating from the masseter region, but the specific quality and word descriptors could assist in differential diagnosis. J Oral Facial Pain Headache 2018;32:418–427. doi: 10.11607/ofph.2042

Keywords: bone surface, glutamate, myofascial pain, subcutaneous, temporomandibular disorders

Myofascial pain is traditionally considered a regional pain syndrome characterized in part by a trigger point in a taut band of skeletal muscle and its associated referred pain. It is generally recognized that myofascial pain is a complex pain disorder and that multiple factors are involved in its underlying pathophysiology. In the craniofacial region, myofascial pain is mainly considered a subset of temporomandibular disorders (TMD), which encompass many of the joint and muscle disorders occurring in the masticatory system. TMD is one of the most common musculoskeletal disorders characterized by persistent or intermittent pain and is associated with a variety of clinical problems, impaired oral health–related quality of life, and psychosocial distress. The myofascial pain in the craniofacial region is not better accounted for by another pain diagnosis.

The etiology and pathophysiology of myofascial pain in the craniofacial region have not been fully elucidated. Numerous biomarkers have been identified and discussed in relation to myofascial TMD pain, and a particular focus has been directed toward the excitatory amino acid glutamate. It has been reported that glutamate levels are significantly higher in painful tendons and muscles compared to pain-free tendons with no signs of clinical inflammation. Elevations in interstitial glutamate levels in muscles may contribute to increased muscle pain sensitivity in TMD. Also, myofascial TMD pain patients show a signifi-
cantly increased level of glutamate in the masseter muscle. This elevation of glutamate is considered to evoke afferent discharges, in part through activation of peripheral N-methyl-D-aspartate (NMDA) receptors. The presence of NMDA receptors has been demonstrated in deep tissues such as muscle, tendon, and bone. Additionally, peripheral NMDA receptors are speculated to be present in skin in association with the terminal endings of nerve fibers.

Injections of small amounts (0.2–0.5 mL) of glutamate (0.2 to 1 M) into the masseter muscle of healthy participants produce pain lasting up to about 10 minutes. This glutamate-evoked masseter muscle pain has similarities to the muscle pain in myofascial TMD patients and may therefore be used as a model of some aspects of myofascial TMD pain. Although glutamate-evoked jaw muscle pain has been extensively investigated in earlier studies, there have been no reports on whether glutamate-evoked pain is altered by the injection depth in the masseter region. All previous studies have relied on clinical examination and administration of the injected solution into the bulkiest part of the masseter muscle, identified during a strong contraction, followed by a needle inserted through the skin and into the muscle with the aim of reaching the mandibular bone and then retracted for 2 to 3 mm before the solution is injected after aspiration. However, no studies have so far contrasted the painful responses to masseter muscle injections with those of superficial injections into the subcutaneous regions or deep injections into the bone surface regions.

Although human experimental muscle pain models may serve as proxies for a better understanding of deep tissue pain and referred pain patterns, the differences and similarities between subcutaneous and bone surface injections in the masseter region need to be established to fully appreciate jaw muscle pain. The aim of this study was therefore to investigate whether glutamate-evoked pain in the masseter region differs between three different depths of glutamate injection, targeting subcutaneous, muscle, and bone tissues.

Materials and Methods

Participants

A total of 16 healthy volunteers (12 men and 4 women, mean ± standard deviation [SD] age: 24.7 ± 4.0 years old) participated in this study. They were recruited by an advertisement posted at Aarhus University (Denmark). None of the participants reported current TMD, which was ascertained according to the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD). Exclusion criteria were: craniofacial pain; serious psychological or physical illness; allergy to glutamate; and/or any chronic illness (eg, uncontrolled hypertension, asthma, diabetes mellitus).

This study was approved by the Central Denmark Region Committees on Biomedical Research Ethics (No.1-10-72-158-16) and performed in accordance with the Helsinki Declaration II. Written informed consent was obtained from all participants, who were informed of their right to withdraw from the study at any time.

Experimental Protocol

This study was performed in a randomized, double-blinded, crossover trial. All subjects participated in three separate sessions, each with a different depth of injection: subcutaneous, intramuscular, or the bone surface layers of the masseter muscles. Each session was separated by an interval of a minimum of 3 days, and the injection order was randomized by a clinical assistant.

In each session, one of the three different depths was estimated in both the right and left masseter muscles (Fig 1). Glutamate or isotonic saline, in a randomized order, was injected into the masseter muscle on one side (left or right) as a noxious stimulus. Perceived levels of pain were estimated intermittently during the experiment using an electronic visual analog scale (eVAS). Quality and locations of pain were estimated intermittently (at 5 and 15 minutes postinjection) using the McGill Pain Questionnaire (MPQ) and pain drawings. Perceived levels of pain, unpleasantness, tiredness, tension, soreness, and stiffness from the injected side of the masseter muscle were also estimated intermittently using a numeric rating scale (NRS) for each measure during the postinjection period. Pressure pain sensitivity (PPS) was also estimated during the experiment intermittently using the NRS. After injection of glutamate or saline on one side, the opposite side was injected with the other solution, and the estimations were repeated (Fig 1).

Noxious Stimuli

Monosodium glutamate (1.0 M, 0.2 mL) (Ajinomoto) or isotonic saline (0.9%, 0.2 mL) was injected into the right or left masseter region over 10 seconds with a 27-G hypodermic needle attached to a disposable syringe. The injection point was set on the most prominent spot of the masseter muscle, which was identified by manual palpation of the muscle by the investigator during tooth clenching by the subject. To imitate superficial vs deep tissue pain, injections to three different depths were performed: (1) subcutaneous (1 to 2 mm below the skin surface); (2) intramuscular (injection performed after reaching contact with the bone surface and retracting the needle 2 to 3 mm); and (3) bone surface (injection...
performed while the needle was in contact with the bone surface). Participants were given careful instructions to keep the jaw relaxed while solutions were injected into the muscle. The injection order of the three sessions was randomized by a clinical assistant. The participants were unaware of injection content and depths.

In five participants, an ultrasound device (Toshiba medical system corporation, Nemio XG SSA-580A) was used to assess and visualize the position of the needle before each injection (Fig 2). For practical reasons and lack of access to the ultrasound device, the ultrasound visualization was not performed in all participants.

**Perceived Levels of Pain**

**Electronic Visual Analog Scale.** The participants were asked to score the ongoing pain intensity on an eVAS. The lower extreme was marked “no pain,” and the upper extreme was marked “most pain imaginable.” The eVAS scores were continuously recorded immediately after each injection for 10 minutes (Fig 1). The peak pain intensity (eVAS peak), the duration of pain (eVAS duration), and the area under the VAS time curve (eVAS AUC) were extracted from the recorded eVAS data.

**Numeric Rating Scales.** In addition to the eVAS scores, the participants were asked to rate the intensity of the pain, unpleasantness, tiredness, tension, soreness, and stiffness on a separate 0–10 NRS for each, with 0 representing no pain (or unpleasantness, tiredness, tension, soreness, or stiffness) and 10 the most pain (etc) imaginable at different time points. The participants scored the maximum of each sensation continuously after each injection (Fig 1).

**MPQ and Pain Drawings.** A Danish or English variant of the MPQ was used to characterize pain quality and determine specific words associated with the pain responses. The pain rating indices (PRI) of the total dimension of pain were calculated according to two-way repeated-measures analysis of variance was used to identify a significant effect. MPQ = McGill Pain Questionnaire; PRI = pain rating indices.

## Table 1

<table>
<thead>
<tr>
<th></th>
<th>Glutamate Subcutaneous</th>
<th>Intramuscular</th>
<th>Bone surface</th>
<th>Saline Subcutaneous</th>
<th>Intramuscular</th>
<th>Bone surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unpleasantness (0–10)</td>
<td>6.9 (0.67)</td>
<td>6.1 (0.7)</td>
<td>6.7 (0.52)</td>
<td>3.7 (0.72)</td>
<td>2.0 (0.56)</td>
<td>2.7 (0.59)</td>
</tr>
<tr>
<td>Tiredness (0–10)</td>
<td>2.4 (0.49)</td>
<td>1.9 (0.69)</td>
<td>2.2 (0.64)</td>
<td>1.4 (0.46)</td>
<td>1.0 (0.39)</td>
<td>0.9 (0.4)</td>
</tr>
<tr>
<td>Tension (0–10)</td>
<td>4.2 (0.68)</td>
<td>5.0 (0.65)</td>
<td>5.3 (0.7)</td>
<td>2.7 (0.76)</td>
<td>1.9 (0.49)</td>
<td>2.6 (0.71)</td>
</tr>
<tr>
<td>Soreness (0–10)</td>
<td>4.4 (0.66)</td>
<td>4.6 (0.55)</td>
<td>4.5 (0.56)</td>
<td>1.9 (0.64)</td>
<td>1.5 (0.44)</td>
<td>1.7 (0.46)</td>
</tr>
<tr>
<td>Stiffness (0–10)</td>
<td>3.1 (0.65)</td>
<td>4.1 (0.6)</td>
<td>4.0 (0.48)</td>
<td>1.8 (0.56)</td>
<td>1.5 (0.4)</td>
<td>1.6 (0.54)</td>
</tr>
<tr>
<td>MPQ (PRI) total (0–77)</td>
<td>18.4 (3.13)</td>
<td>18.4 (3.46)</td>
<td>20.8 (2.9)</td>
<td>11.69 (3.1)</td>
<td>7.56 (1.8)</td>
<td>10.38 (2.48)</td>
</tr>
</tbody>
</table>

Two-way repeated-measures analysis of variance was used to identify a significant effect. MPQ = McGill Pain Questionnaire; PRI = pain rating indices.

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Significant difference among injection depths.

Significant difference between glutamate and isotonic saline solutions. Two-way repeated-measures analysis of variance was used to identify a significant effect. MPQ = McGill Pain Questionnaire; PRI = pain rating indices.

Table 1  Mean ± Standard Error (SE) Numeric Rating Scale Scores of Postinjection Symptoms by Injection Depth and Content

<table>
<thead>
<tr>
<th>Injection Depth</th>
<th>Solution</th>
<th>Stiffness</th>
<th>Soreness</th>
<th>Tension</th>
<th>Unpleasantness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subcutaneous</td>
<td>Glutamate</td>
<td>3.1 (0.65)</td>
<td>4.4 (0.66)</td>
<td>4.2 (0.68)</td>
<td>6.9 (0.67)</td>
</tr>
<tr>
<td>Intramuscular</td>
<td>Glutamate</td>
<td>4.1 (0.6)</td>
<td>4.6 (0.55)</td>
<td>5.0 (0.65)</td>
<td>6.1 (0.7)</td>
</tr>
<tr>
<td>Bone surface</td>
<td>Glutamate</td>
<td>4.0 (0.48)</td>
<td>4.5 (0.56)</td>
<td>5.3 (0.7)</td>
<td>6.7 (0.52)</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>Isotonic saline</td>
<td>1.8 (0.56)</td>
<td>1.9 (0.64)</td>
<td>2.7 (0.76)</td>
<td>3.7 (0.72)</td>
</tr>
<tr>
<td>Intramuscular</td>
<td>Isotonic saline</td>
<td>1.5 (0.4)</td>
<td>1.5 (0.44)</td>
<td>1.9 (0.49)</td>
<td>2.0 (0.56)</td>
</tr>
<tr>
<td>Bone surface</td>
<td>Isotonic saline</td>
<td>1.6 (0.54)</td>
<td>1.7 (0.46)</td>
<td>2.6 (0.71)</td>
<td>2.7 (0.59)</td>
</tr>
</tbody>
</table>

ANOVA and post hoc analysis (Tukey HSD) were performed for comparisons. A value of $P < .05$ was considered statistically significant.

Pressure Pain Sensitivity. Three palpometers (5, 10, and 20 N) were used to assess PPS. The tip of the palpometer was percutaneously applied to the masseter at four sites (Fig 3) around the injection site, and the participants were asked to rate the perceived pressure pain on a 0–100 NRS where 0 indicated no pain at all, 50 indicated just barely painful, and 100 indicated the most pain imaginable. Each assessment took approximately 2 seconds. During the pressure stimulation, the participants were instructed to keep their jaw relaxed and teeth slightly apart (without occlusal contacts) with minimal voluntary contraction and to focus their attention on the experimental task (Fig 1).

Statistical Analyses

Two-way repeated-measurements analysis of variance (ANOVA) was used to test differences in eVAS peak pain, eVAS pain duration, eVAS area under the curve (AUC), area of pain drawing, and MPQ score with the factors solution (isotonic saline and glutamate) and injection depth (subcutaneous, intramuscular, and bone surface layer). Two additional three-way repeated-measures ANOVAs were used to test the differences in the PPS (isotonic saline and glutamate) with the factors injection depth (subcutaneous, intramuscular, and bone surface layer), time (baseline, 5, and 15 minutes after injection), and palpometer force (5, 10, and 20 N). McNemar test for paired data was used to test differences in MPQ words chosen by participants. Tukey Honest Significant Difference (HSD) test with correction for multiple comparisons was used for post hoc analysis when appropriate. All results are presented as mean ± standard error of the mean (SEM). Values of $P < .05$ were considered statistically significant.

Results

The position of the needle and the volume effects following injection of isotonic saline could readily be identified on the ultrasound images in the five participants who received ultrasound (Fig 2). Therefore,
all subsequent injections were performed based on clinical guidelines and techniques to target the three different levels in the masseter region.

**Experimental Pain and Other Postinjection Symptoms**

Experimental pain evoked by glutamate injection was scored significantly higher than the experimental pain evoked by isotonic saline injection on all eVAS parameters (peak pain, pain duration, and AUC), regardless of injection depth (Fig 4; Tukey \( P < .021 \)).

Regarding the main effect of injection depth, subcutaneous injections induced significantly more pain than intramuscular injections, as shown for the eVAS peak pain and the eVAS AUC (Figs 4a and 4b; Tukey \( P < .028 \)). The peak pain after isotonic saline injection in the subcutaneous layer was significantly higher than the peak pain evoked by the saline injection into the intramuscular and bone surface layers (Tukey \( P < .007 \)), but there was no significant difference in glutamate-evoked pain between depths (Tukey \( P > .057 \)).

All glutamate injections resulted in areas on pain drawings that were significantly larger than those drawn after the isotonic saline injections at the corresponding depth (Fig 4d; Tukey \( P < .005 \)). Nevertheless, there was no significant effect of depth of injection on pain area for either type of injection.

The results of other postinjection symptoms evoked by glutamate or isotonic saline at the different injection depths are shown in Table 1. The glutamate injections produced significantly higher scores
The main effects of injection depth, time, and force were shown in Table 3 and Fig. 5. For the glutamate injection, the ANOVA analyses of the PPS scores are shown significantly more often than the intramuscular glutamate injection (Table 2; McNemar $P < .026$). The glutamate injection was expressed significantly more often as “pricking” and “annoying” significantly more often than both the subcutaneous glutamate injection and as “pinching” significantly more often than the subcutaneous glutamate injection and as “sharp” significantly more often than the intramuscular glutamate injection and as “pinching” significantly more often than both the intramuscular and bone surface glutamate injections (Table 2; McNemar $P < .046$). The intramuscular glutamate injection was expressed significantly more often as “boring” than the subcutaneous glutamate injection (Table 2; McNemar $P < .026$). The glutamate injection at the surface of the bone was described as “cramping” significantly more often than the subcutaneous glutamate and the bone surface isotonic saline injections and as “penetrating” significantly more often than the intramuscular glutamate injection (Table 2; McNemar $P < .046$). The intramuscular saline injection was described as “pricking” and “annoying” significantly more often than the intramuscular glutamate injection (Table 2; McNemar $P < .026$).

## Pressure Pain Sensitivity

The ANOVA analyses of the PPS scores are shown in Table 3 and Fig. 5. For the glutamate injection, the main effects of injection depth, time, and force were than isotonic saline injections for all symptoms (unpleasantness, tiredness, tension, soreness, stiffness, and MPQ scores) ($F > 4.667$, $P < .048$). There was no significant effect of depth of glutamate injection on tiredness, tension, soreness, stiffness, or MPQ scores. Subcutaneous injections (glutamate and isotonic saline) were rated as significantly more unpleasant than intramuscular injections ($F > 4.866$, $P < .015$; Tukey: $P < .011$).

The comparisons of the MPQ words chosen by more than five participants for each injection and depth are shown in Table 2. Although there were no depth-related differences in NRS scores of muscle symptoms, the subcutaneous glutamate injection was expressed as “sharp” significantly more often than the intramuscular glutamate injection and as “pinching” significantly more often than both the intramuscular and bone surface glutamate injections (Table 2; McNemar $P < .046$). The intramuscular glutamate injection was expressed significantly more often as “boring” than the subcutaneous glutamate injection (Table 2; McNemar $P < .026$). The glutamate injection at the surface of the bone was described as “cramping” significantly more often than the subcutaneous glutamate and the bone surface isotonic saline injections and as “penetrating” significantly more often than the intramuscular glutamate injection (Table 2; McNemar $P < .046$). The intramuscular saline injection was described as “pricking” and “annoying” significantly more often than the intramuscular glutamate injection (Table 2; McNemar $P < .026$).

### Table 2 Comparisons Among Words from McGill Pain Questionnaire Chosen by More Than Five Participants (30%) for Each Injection and Depth (n = 16)

<table>
<thead>
<tr>
<th></th>
<th>Subcutaneous</th>
<th>Intramuscular</th>
<th>Bone surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sharp</td>
<td>8$^a$</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Pinching</td>
<td>6$^{a,b}$</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Boring</td>
<td>0$^a$</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Taut</td>
<td>3</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Cramping</td>
<td>0$^a$</td>
<td>3</td>
<td>5$^c$</td>
</tr>
<tr>
<td>Penetrating</td>
<td>3</td>
<td>1$^d$</td>
<td>5</td>
</tr>
<tr>
<td>Pricking</td>
<td>5</td>
<td>1$^e$</td>
<td>1</td>
</tr>
<tr>
<td>Tender</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Annoying</td>
<td>4</td>
<td>0$^a$</td>
<td>1</td>
</tr>
<tr>
<td>Tight</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

McNemar test was used for all statistical comparisons.

- $^a$Significant difference between subcutaneous glutamate injection and intramuscular glutamate injection ($P < .026$).
- $^b$Significant difference between subcutaneous glutamate injection and bone surface glutamate injection ($P < .046$).
- $^c$Significant difference between bone surface glutamate injection and isotonic saline bone surface injection ($P < .026$).
- $^d$Significant difference between subcutaneous glutamate injection and intramuscular glutamate injection ($P < .026$).
- $^e$Significant difference between intramuscular saline injection and intramuscular glutamate injection ($P < .026$).

### Table 3 Results of Analysis of Variance of Injection Depth, Measurement Time, and Palpometer Simulation Force ($P$ Value, $F$ Value)

<table>
<thead>
<tr>
<th></th>
<th>Depth</th>
<th>Time</th>
<th>Force</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamate</td>
<td>.003</td>
<td>&lt; .001</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Saline</td>
<td>NS</td>
<td>&lt; .001</td>
<td>&lt; .001</td>
</tr>
</tbody>
</table>

$NS = $ not statistically significant. |Baseline, 5 minutes postinjection, and 15 minutes postinjection.

The comparisons of the MPQ words chosen by more than five participants for each injection and depth are shown in Table 2. Although there were no depth-related differences in NRS scores of muscle symptoms, the subcutaneous glutamate injection was expressed as “sharp” significantly more often than the intramuscular glutamate injection and as “pinching” significantly more often than both the intramuscular and bone surface glutamate injections (Table 2; McNemar $P < .046$). The intramuscular glutamate injection was expressed significantly more often as “boring” than the subcutaneous glutamate injection (Table 2; McNemar $P < .026$). The glutamate injection at the surface of the bone was described as “cramping” significantly more often than the subcutaneous glutamate and the bone surface isotonic saline injections and as “penetrating” significantly more often than the intramuscular glutamate injection (Table 2; McNemar $P < .046$). The intramuscular saline injection was described as “pricking” and “annoying” significantly more often than the intramuscular glutamate injection (Table 2; McNemar $P < .026$).

The main effects of injection depth, time, and force were shown significantly more often than the intramuscular glutamate injection (Fig 5a; McNemar $P < .026$). The glutamate injection significantly increased compared to baseline (Fig 5a; Tukey $P < .003$). The post hoc analysis of the main effect of time indicated that the PPS scores 5 minutes after injection were statistically higher than the PPS scores evoked after the intramuscular glutamate injection (Fig 5a; Tukey $P < .003$). The post hoc analysis of the main effect of time indicated that the PPS scores 5 minutes after injection were no longer different (Fig 5a; Tukey $P = .994$). The post hoc analysis of the main effect of force indicated that the PPS scores at force 20 N were significantly higher than at 5 N and 10 N (Tukey $P < .001$), and the PPS scores at force 10 N were significantly higher than at 5 N (Tukey $P < .001$).

For the isotonic saline injection, there was no significant main effect of injection depth ($P = .970$), but the main effects of injection time and force were significant (Table 3; $P < .001$). There were no statistical interactions between factors ($P > .439$). The post hoc analysis of the main effect of time indicated that the PPS score 15 minutes after injection was
significantly decreased compared to baseline and 5 minutes after injection (Fig 5b; Tukey P < .002). The post hoc analysis of the main effect of force indicated that the PPS scores at 20 N were significantly higher than at 5 N and 10 N (Tukey P < .001) and that the PPS scores at 10 N were significantly higher than at 5 N (Tukey P < .001).

**Discussion**

The present study has shown that the depth of injection into the masseter region affected the characteristics of experimental pain and postinjection reactions. In particular, a significant finding was that the PPS scores after glutamate injection were significantly affected by injection depth and that, overall, the subcutaneous injection (for both glutamate and isotonic saline) was more painful and unpleasant than the intramuscular injection. The PPS scores after glutamate injection at the surface of the bone were significantly higher than those after intramuscular glutamate injection, while the PPS scores after isotonic saline injection were not significantly affected by injection depth. These findings lead to two suggestions: First, the subcutaneous injection was more painful and unpleasant than the intramuscular injection. Second, bone surface, but not intramuscular glutamate injections, were able to modulate the PPS of the masseter region.

**Injection Pain and Postinjection Reactions**

Before the present study, glutamate has most often been injected intramuscularly when used as a pain model. No previous studies in the craniofacial region have systematically evaluated and compared the effect of injection depth. Unexpectedly, there were no significant differences between the effects of each depth of glutamate injection on the eVAS parameters (peak of pain, duration, and AUC) in the present study. If it is assumed that injection of glutamate into the masseter muscle evokes afferent discharges through activation of peripheral NMDA receptors, these results suggest that the expression of peripheral NMDA receptors are equally distributed in different craniofacial tissues. The expression of glutamate receptors has been recognized on the unmyelinated terminal endings of cutaneous afferent fibers from the trunk and arm of humans. Additionally, a previous study has indicated that following injection of glutamate into the human forehead skin, cutaneous pain responses are evoked and subjects become sensitive to mechanical stimulation.

On the other hand, when considering both glutamate and isotonic saline injection scores together, subcutaneous injections induced higher eVAS peak pain and larger AUC and unpleasantness scores than intramuscular injections. It may be speculated that injection of a bolus into the dense subcutaneous tissue as compared to intramuscular injection caused increased mechanical nociceptor excitement because the subcutaneous glutamate injection was significantly more often rated as “sharp” and “pinching” than the intramuscular glutamate injection. In the present study, there was no significant effect of the glutamate injection depth on the spatial distribution of pain, evaluated through areas of pain drawings. Taking into account that there are differences in histologic density and penetration of the injection solution...
in each of three layers, such a difference between injection depths in terms of area of pain drawing could have been expected. However, in a previous study, the pain area expressed as a percentage of the total body area was correlated with pain intensity.\(^3^6\) In general, changes in pain area are less sensitive to alterations in pain intensity than other measures.\(^2^7\) Hence, the present results support the view that the amount of change in the injection-related pain scores was insufficient to alter the perceived area of pain, in accordance with a previous glutamate injection study.\(^2^7\)

**PPS and Deep Pain Sensation**

PPS scores in muscles and joints assessed by manual palpation are regarded as an important clinical finding of TMD and other musculoskeletal pain conditions.\(^3^7\) The palpometer used in the present study has low test-retest variability and provides a more accurate pressure stimulus than manual palpation\(^3^1\) at the stimulus intensities suggested by the DC/TMD.\(^4\) A number of previous studies have reported that TMD pain patients have higher pressure sensitivity of their masticatory muscles than asymptomatic individuals.\(^3^8^\text{-}4^0\) In a previous study, the pressure sensitivity assessment suggested that injection of 0.5 mol/L glutamate into the masticatory muscle may model myofascial TMD pain as effectively as higher concentrations of glutamate.\(^2^0\)

Although some studies involving simple intramuscular glutamate injections into the masseter muscle have been carried out, significant increases in pressure sensitivity, as in clinical TMD patients, have so far generally not been detected using palpometers.\(^1^9,2^5\) On the other hand, a high-temperature acidic glutamate (pH 4.8, 48°C) injection into the masseter muscle caused significant increases in pressure sensitivity.\(^2^8\) In the present study, differences in PPS assessed with 5-N and 10-N palpometers were observed between before and after the glutamate injection at the surface of the bone, but not for the other two sites of injection. Moreover, the PPS scores evoked by the glutamate injection at the surface of the bone were significantly higher than the PPS scores evoked by intramuscular glutamate. It is especially noteworthy that the differences were observed using all force levels (5 N, 10 N, 20 N). These results suggest that the glutamate injection at the surface of the bone sensitized the deep tissues to pressure stimulation.

Previous studies have suggested that intramuscular glutamate injection into the masseter muscle excites and mechanically sensitizes nociceptors through activation of NMDA receptors.\(^1^6,3^3\) In the present study, a bone surface injection with the needle tip in direct contact with the bone surface was performed. Because the periosteum contains a higher density of nociceptors than the cortical bone, it was hypothesized that injection at the surface of the bone would excite nociceptors in the periosteum.\(^4^1\) Also, glutamate bone surface injection may have caused inflammation and/or an increase in intraosseous pressure that may have activated peripheral sensory nerve terminals within the bone marrow through the release of inflammatory mediators and/or by mechanical compression or distortion.\(^4^2\) Furthermore, the sensitization to deep pressure stimulation following glutamate injection at the surface of the bone was most likely due to the action of glutamate, as this sensitization was not seen following isotonic saline injections. It can be suggested that the polymodal nociceptors in the periosteum were excited by the injected glutamate and sensitized to pressure pain. Polymodal nociceptors dominated by C fibers are prone to sensitization, so they are considered to be important for the development of pain in the temporomandibular joint region.\(^4^3,4^4\) In the present study, the glutamate injection at the surface of the bone was described as “taut,” “cramping,” and “penetrating.” These pain descriptors may represent the second pain caused by excitation of C fibers through the polymodal nociceptors.\(^4^5\)

**Methodologic Considerations**

The design of the present study is considered a major strength and advantage because sequence effects and potential bias from participants and examiners can be ruled out. Isotonic saline was used as a placebo control, but in accordance with previous studies,\(^7,1^0,1^6,2^0,2^5,2^7\) injections of isotonic saline are not completely pain-free. The pain and associated responses evoked by the isotonic saline injections could be due to nonspecific volume effects. Perhaps isotonic saline injections should not be considered a true placebo, but rather a nonspecific, low-level noxious input. A limitation of this study was that gender effects could not be examined due to the low sample size and lack of statistical power. Previous studies with intramuscular injections of glutamate have indicated significantly higher pain scores in women than in men,\(^1^6,1^9,2^4\) but no differences in the degree of sensitization to mechanical stimulation.\(^1^9\) The present study can be used to design future studies with a specific focus on gender differences in pain sensitivity across different craniofacial tissues. Finally, it should also be emphasized that the needle position and injection depth were only confirmed in pilot experiments in a few participants. The main experiment relied on the clinical procedure and insertion of the needle into different layers of the masseter region; however, the standard use of ultrasound for all injections was not always possible and not deemed essential; clinically, it seemed evident that injections were located in the subcutaneous tissue, muscle tissue, or close to the
periosteum. Obviously, it cannot be completely ruled out that the injected solution could have leaked from the deeper sites, but the ultrasound images indicated a fairly well localized deposit of fluid in the appropriate sites. Overall, it is argued that the present study design and methodology allow conclusions to be drawn about differences and similarities in pain characteristics following a systematic variation in targeted injections of three sites in the masseter region.

Conclusions

The present study suggests that glutamate-evoked symptoms in the masseter region were altered by the three different depths of glutamate injection targeting subcutaneous, intramuscular, and bone surface regions. Subcutaneous injections were more painful and unpleasant than intramuscular injections. Although injection at the bone surface and intramuscular injection caused similar pain intensity, duration, and area, the findings indicated that glutamate injection at the surface of the bone may have sensitized the deep pain tissues to pressure stimulation. This sensitization was not evoked by the isotonic saline injection or the intramuscular glutamate injection. These findings indicate that further studies of deep pain sensitization variations in glutamate injection depths in the masseter region may lead to a further understanding of myofascial TMD pain. In particular, it can be suggested that while it is difficult in the clinic to differentiate between the source or site of pain originating from the masseter region, the specific quality and word descriptors could assist in the differential diagnosis.

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