Systematic Mapping of Pressure Pain Thresholds of the Masseter and Temporalis Muscles and Assessment of Their Diversity Through the Novel Application of Entropy

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Aims: To assess the diversity of pressure pain thresholds (PPTs) within the masseter and temporalis muscles by using the novel concept of entropy and to assess the differences in PPT scores between different sites of the masseter and temporalis muscles. **Methods:** In this randomized, single-blinded study, the left and right masseter and temporalis muscles of 14 healthy volunteers were divided into 15 sites each, and the PPT was assessed for each of these sites. PPT assessments were performed in two different sessions. Entropy and center of gravity (COG) values were calculated for the PPTs of each muscle. Repeated measures analysis of variance was used to assess differences between muscles, sides, and sites for PPT, entropy, and COG scores. **Results:** The main findings were: *(1)* PPT scores varied significantly between the masseter and temporalis muscles and within each of these muscles; *(2)* entropy values of PPT scores were not different between the masseter and temporalis muscles; and *(3)* COG values of PPT scores varied statistically, but these changes do not seem to be clinically relevant. **Conclusion:** The results of this study suggest that the anatomical layout of the masseter and temporalis muscles has implications for mechanical pain sensitivity and that areas have different sensitivities within these muscles. Furthermore, reference values for the entropy of PPTs in healthy individuals have been estimated, and comparing these values with those of patients with muscle-related pain conditions can provide quantitative information about the spatial heterogeneity of mechanical pain sensitivity, which may be a valuable clinical outcome measure. *J Oral Facial Pain Headache 2017;31:362–371. doi: 10.11607/ofph.1927*

Keywords: *entropy, mechanical pain sensitivity, palpation, pressure pain thresholds, quantitative sensory test*

Muscle tenderness and pain on palpation are some of the most common findings in several pain conditions, including temporomandibular disorders (TMD),[1] tension-type headache,[2,3] and migraine.[4] One of the most common metrics used to assess mechanical pain sensitivity (MPS) of the musculoskeletal tissues is pressure pain threshold (PPT), which has been shown to be altered (ie, decreased) in a variety of pain conditions.[5] The PPT is a quantitative sensory test and is usually determined by a device such as an algometer with which increasing pressure is applied until the participant feels that the pressure has become painful.

Even though PPTs are commonly used in the assessment of patients with TMD and headaches, reference values to distinguish healthy from pathologic individuals are currently not available, despite several studies.[6,7] Increasing evidence of functional compartmentalization of the masseter[8–10] and temporalis muscles[11] and differences in innervation within these muscles pose the question of whether pain perception varies depending on which part of the muscle is examined and/or affected by nociceptive inputs. These variations may explain the different values that have been obtained among the mentioned studies, as the assessment sites were possibly not the same.

It has been proposed that the diversity of pain as a variable should be considered to enrich the characterization of different diagnoses of myofascial pain.[12,13] Since reference values for PPTs are not available, it may be that the amount of diversity of mechanical sensitivity

within each muscle could serve as a reference value for healthy individuals. Only a few studies have attempted to assess the diversity of the masticatory muscles by mapping the PPTs of different sites within the masseter and temporalis muscles[14] or in the temporalis muscle alone.[15,16] These studies found site-to-site differences within each muscle[14–16] and a decrease in PPT from the posterior to anterior temporalis muscle for headache patients but not for healthy controls.[15,16] Despite the systematic approaches of these studies, there were areas of the temporalis and masseter muscles that were not assessed, thus leaving unanswered the question of how diverse the PPT scores of these muscles truly are. Furthermore, while statistically comparing PPT scores of different sites and regions of a muscle is important, this does not provide a clear understanding of the amount of PPT diversity among all assessed sites, as it only reveals if there are differences from each muscle site to another.

In addition to other entropies, the concept of Shannon entropy[17] has been used in biology as an index to assess diversity of species.[18] A higher entropy value indicates more diversity, and a lower entropy value indicates less diversity. Entropy has been used previously to assess diversity of mechanical sensitivity[19]; however, in that study, PPT values were not assessed. Instead, standardized forces were applied to the masseter muscle. Furthermore, an experimental pain model was used, and only the masseter muscle was assessed for entropy. With this in mind, an entropy index that was introduced recently was applied[19] to determine the diversity of PPT values obtained from a systematic assessment of the masseter and temporalis muscles.

The aims of this study were to assess the diversity of PPT scores within the masseter and temporalis muscles by using the novel concept of entropy and to assess the differences in PPT scores between different sites of the masseter and temporalis muscles. The results of the present study may provide reference values for diversity of mechanical pain sensitivity in healthy individuals and allow the pressure pain sensitivity of the entirety of both the masseter and temporalis muscles to be mapped for the first time.

Materials and Methods

Participants

A total of 14 healthy volunteers between the ages of 21 and 53 years (mean ± standard deviation [SD]; 34.1 ± 9.1; 7 men and 7 women) were recruited and assessed between April and June of 2014 in the laboratory of the Section of Orofacial Pain and Jaw Function, Department of Dentistry, HEALTH, Aarhus University. These participants were a different set of participants than the ones included in a recent entropy study.[19] The timeline of the study is described in Fig 1a. The inclusion criteria were: no medical history of systemic diseases, pregnancy, use of medications (eg, nonsteroidal anti-inflammatory drugs, muscle relaxants, anxiolytics, or hypnotics), orofacial pain, or temporomandibular pain symptoms (assessed with a TMD pain screener[20]). The study protocol followed the guidelines of the World Medical Association Declaration of Helsinki and was approved by the Midtjylland regional ethical committee. The participants signed an informed consent document and agreed to participate in the study after being provided written and oral information about the experiment.

Study Design

The study was performed as a randomized, single-blinded study. Assessment of PPTs was done in two different sessions at least 5 days apart (Fig 1). The order in which muscles, sides, and sites were assessed was randomized using randomization.com. All assessments were performed for all participants.

PPT Scores of the Temporalis and Masseter Muscles

The anterior-posterior (AP) and superior-inferior (SI) borders of both the right and left temporalis and masseter muscles were identified by palpation while the participants actively clenched their teeth. These borders were used as landmarks to divide the muscles into 15 sites (3 vertical × 5 horizontal for the masseter muscle and 5 vertical × 3 horizontal for the temporalis muscle). These sites were marked on the skin with a skin pencil and were numbered 1 through 15 for both the right and left masseter muscles and the right and left temporalis muscles (Fig 1b). For analysis purposes, the area of the masseter muscle was further divided into five SI regions and three AP regions, and the temporalis was divided into five AP regions and three SI regions.

A handheld electronic pressure algometer (Somedic Production AB) was used to assess the PPT at each of the 15 sites on all four muscles. This was done by applying increasing forces with an algometer (which was fitted with a rubber tip probe that had a circular surface area of 1 cm^2) perpendicularly to the skin at a continuous rate of approximately 30 kPa/second. The rate was visualized in a digital readout of ramp rate, and the force was applied until the participants indicated the perception of pain by pressing on a button connected to the algometer. If needed, the hair of the participant was manually moved aside enough that the site marks could be seen on the skin. The PPT score was reported in units of pressure (kPa). All participants were examined in a

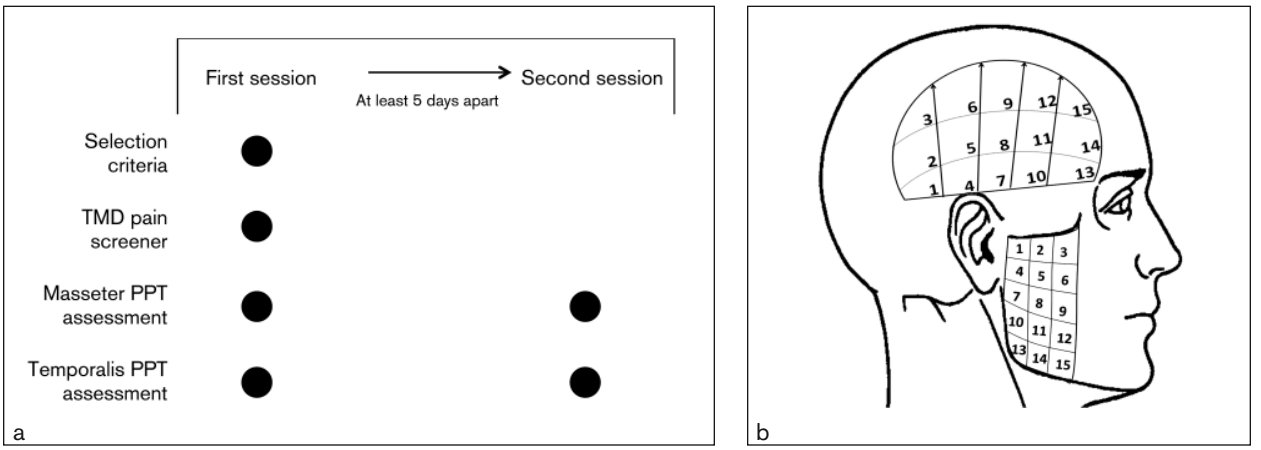

Fig 1 Timeline of study and site numbers. **(a)** The pressure pain thresholds (PPTs) were assessed for all 15 sites in two different sessions. **(b)** The anterior-posterior and inferior-superior borders of the masseter and temporalis muscles were identified, and the areas were divided into 15 sites.

dental chair, sitting upright with the head resting in a comfortable and neutral position. One measurement of PPT for each site in each session was taken to avoid muscle fatigue and site overlapping. The same protocol was applied during the second session after a minimum interval of 5 days. Each session took approximately 1 hour per subject. To verify that the participants had understood the explanations and were familiarized with the protocol, the procedures were tested in the hypothenar eminence three times before the first session. All participants were examined, and all the experimental sessions were performed by the same examiner (A.M.).

Entropy of Mechanical Pain Sensitivity

Entropy is a measure of diversity of values. Entropy for a particular experimental condition with a set of M possible outcomes is highest if all values have maximum diversity, and the minimum value of entropy is 0 if all outcomes are equal. In the context of the 15 PPT scores of each muscle, entropy indicates the degree of diversity of those 15 scores, with higher entropy values corresponding to more diverse PPT scores; ie, greater heterogeneity over the grid. Entropy was calculated in Excel from the PPT scores of the 15 sites within each muscle (right and left masseter and temporalis muscles) and for each session. The maximum entropy possible for this set-up was 2.708.

Center of Gravity of Mechanical Pain Sensitivity

To evaluate the spatial distribution of the mechanical sensitivity in the masseter and temporalis muscles, the center of gravity (COG) calculation technique based on the principles described by Thygesen et al[21] was used. The COG coordinates were defined as Σx_i*gridvalue$_i$/Σgridvalue$_i$; Σy_i*gridvalue$_i$/Σgridvalue$_i$. The PPT scores from each of the 15 sites in each muscle were used as the grid values. The weighting of the PPT scores in this template enabled the creation of a representational point of the "center" of PPT values in quantitative terms. The two coordinates of the center of the PPT (coordinates x and y from the lateral-medial and from the superior-inferior direction of the masseter; x and y axes from the posterior-anterior and y axis from the inferior-superior direction of the temporalis) were determined. Furthermore, a graphical representation of the COG of each muscle was plotted to determine any changes in COG among the plotted muscles.

Data Analyses

Statistical analyses were run in Statistica (version 5.1). Reliability of PPT scores between the two sessions was assessed by using intraclass correlation coefficient (ICC). The calculations were based on a mean-rating, absolute agreement, two-way, mixed-effects model. The ICC was calculated for each of the four assessed muscles separately.

Repeated measures analysis of variance (ANOVA) was used to test for:

- Differences in PPT scores between the masseter and temporalis muscles by using gender (2 levels), session (2 levels), and muscle (2 levels) as factors
- Differences in PPT scores within regions of the masseter muscle by using gender (2 levels), session (2 levels), side (2 levels), AP region (3 levels), and SI region (SI) (5 levels) as factors
- Differences in PPT scores within regions of the temporalis muscle by using gender (2 levels), session (2 levels), side (2 levels), SI region (3 levels), and AP region (5 levels) as factors

- Differences in PPT scores between sites within the masseter muscle by using gender (2 levels), session (2 levels), side (2 levels), and site (15 sites) as factors
- Differences in PPT scores between sites within the temporalis muscle by using gender (2 levels), session (2 levels), side (2 levels), and site (15 sites) as factors

The entropy and COG coordinate values (x and y coordinates tested separately) were analyzed by using ANOVA with the following factors: gender (2 levels), session (2 levels), muscle (2 levels), and side (2 levels). ANOVA analyses of PPT were done separately for the masseter and temporalis muscles because the regions and sites of these muscles cannot be compared. Also, both main factors and higher-level interactions were interpreted, as in these analyses the main effects are not being driven by the interactions. Tukey post hoc tests were used to adjust for multiple comparisons to avoid type I error. Pearson correlation was used to test for correlations between entropy and age and between PPT and age. For all tests, the significance level was set at $P < .05$. Mean values are reported in the text and figures.

Results

PPT Scores

There was a significant overall difference in PPT scores between the temporalis and masseter muscles ($P < .001$; 3-way ANOVA) (Fig 2). The mean PPT score for the temporalis muscle was 211.7 kPa and was 151.0 kPa for the masseter muscle. There were no other significant differences.

Reliability. PPT showed good to excellent reliabilty among the mean measurements for each of the sides and muscles. The right masseter had an ICC of 0.89, the left masseter 0.95, the right temporalis 0.91, and the left temporalis 0.85.

Masseter Muscle. ANOVA analyses showed an overall significant difference between the AP and SI regions within the masseter muscle and significant interactions for AP region × SI region (F = 2.72) and session × side × AP region (F = 3.57) ($P < .01$; 5-way ANOVA). As shown in Figs 3b and 3d, there was a significant difference between the anterior, middle, and posterior regions of the masseter muscle ($P < .001$; 5-way ANOVA), with the post hoc analysis showing that all regions were significantly different from each other ($P < .05$) (Fig 3d). Furthermore, the posterior region of the masseter muscle exhibited the highest PPT (mean = 166.4 kPa), and the anterior region of the masseter exhibited the lowest PPT (mean = 138.6 kPa). There was a significant difference between regions in the SI aspect of the masseter muscle ($P < .001$; 5-way ANOVA) (Figs 3a and 3c). The post hoc analysis showed a significant difference between the superior aspect of the masseter muscle and all other regions in the SI aspect ($P < .01$) and also between the superior-middle (SM) region and the inferior region ($P < .01$) (Fig 3c). ANOVA also showed a significant overall difference between the 15 sites within the masseter muscle. The site within the masseter with the highest PPT was site 1 (mean = 195.1 kPa), and the site with the lowest was site 9 (mean = 129.8 kPa) (Fig 1b). Site 1 showed a significantly higher PPT than all other sites ($P < .05$; Tukey post hoc). There were no other significant differences.

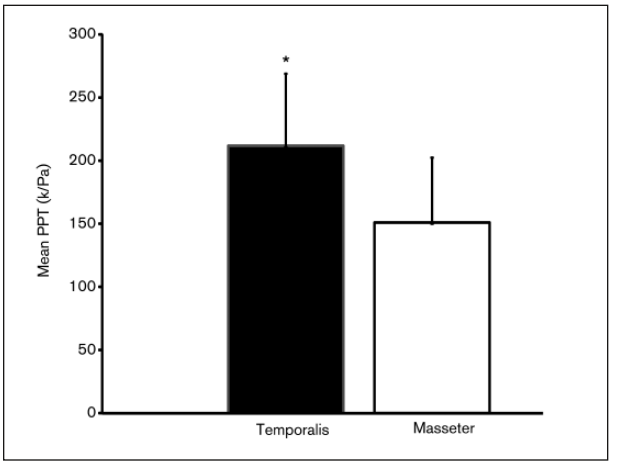

Fig 2 Mean pressure pain thresholds (PPTs) of masseter and temporalis muscles. Significantly higher PPT scores were seen for the temporalis compared to the masseter muscle. (*$P < .001$; 3-way ANOVA).

Temporalis Muscle. As shown in Figs 4a and 4c, ANOVA demonstrated a significant difference between the AP regions of the temporalis muscle, ($P < .001$; 5-way ANOVA) and there were significant interactions for gender × session × SI region (F = 5.92) and gender × session × side × SI region (F = 4.22). Post hoc analysis showed that all AP regions were significantly different from each other ($P < .05$), with the exception of the posterior region compared to the posterior-middle (PM) region ($P = .39$) and the PM region compared to the middle region ($P = .69$) (Figs 4b and 4d). Moreover, ANOVA showed a significant overall difference between all 15 sites within the temporalis muscle. The site within the temporalis muscle with the highest PPT was site 2 (mean = 237.5 kPa), and the one with the lowest was site 14 (mean = 177.9 kPa) (Fig 1b).

Entropy Values

ANOVA did not show any significant differences in entropy values. The mean entropy value for the

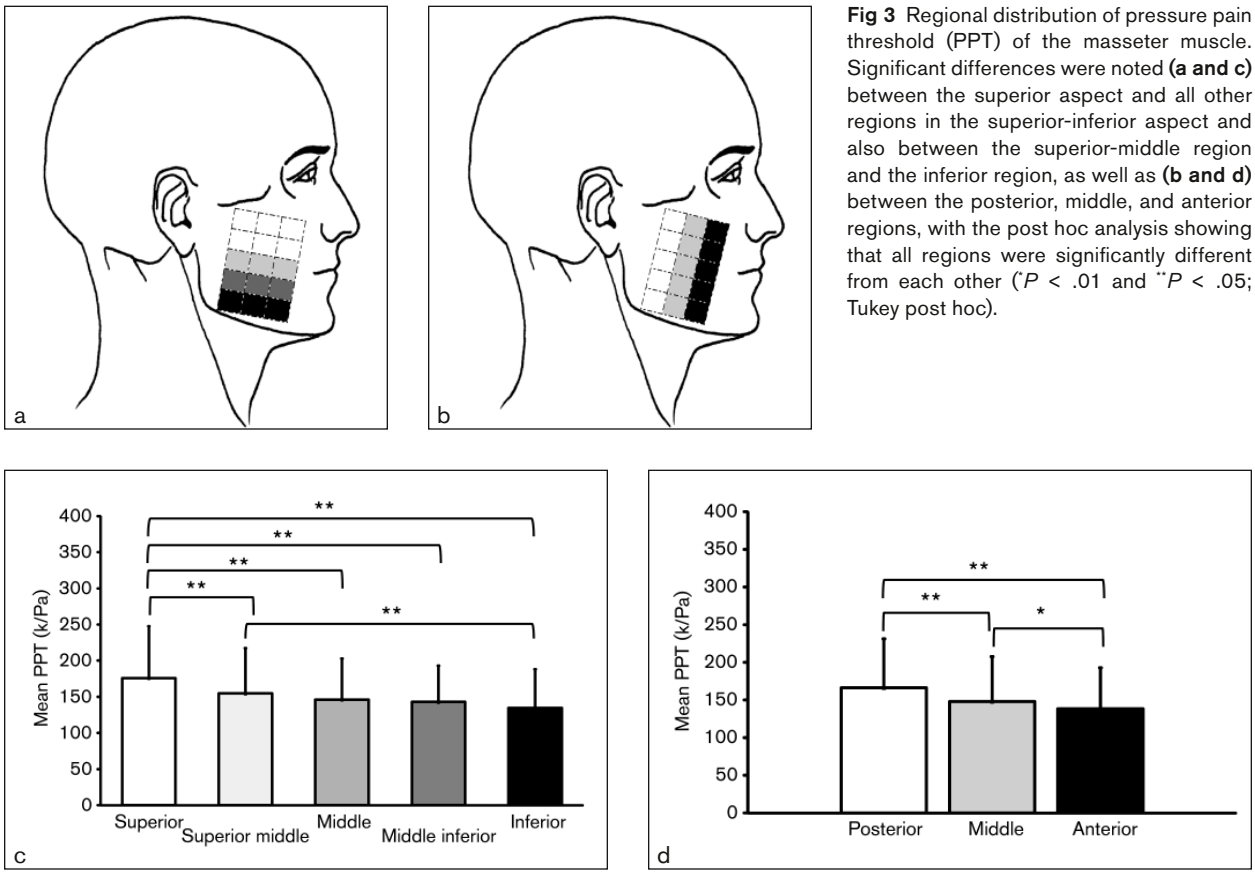

Fig 3 Regional distribution of pressure pain threshold (PPT) of the masseter muscle. Significant differences were noted **(a and c)** between the superior aspect and all other regions in the superior-inferior aspect and also between the superior-middle region and the inferior region, as well as **(b and d)** between the posterior, middle, and anterior regions, with the post hoc analysis showing that all regions were significantly different from each other ($^{*}P < .01$ and $^{**}P < .05$; Tukey post hoc).

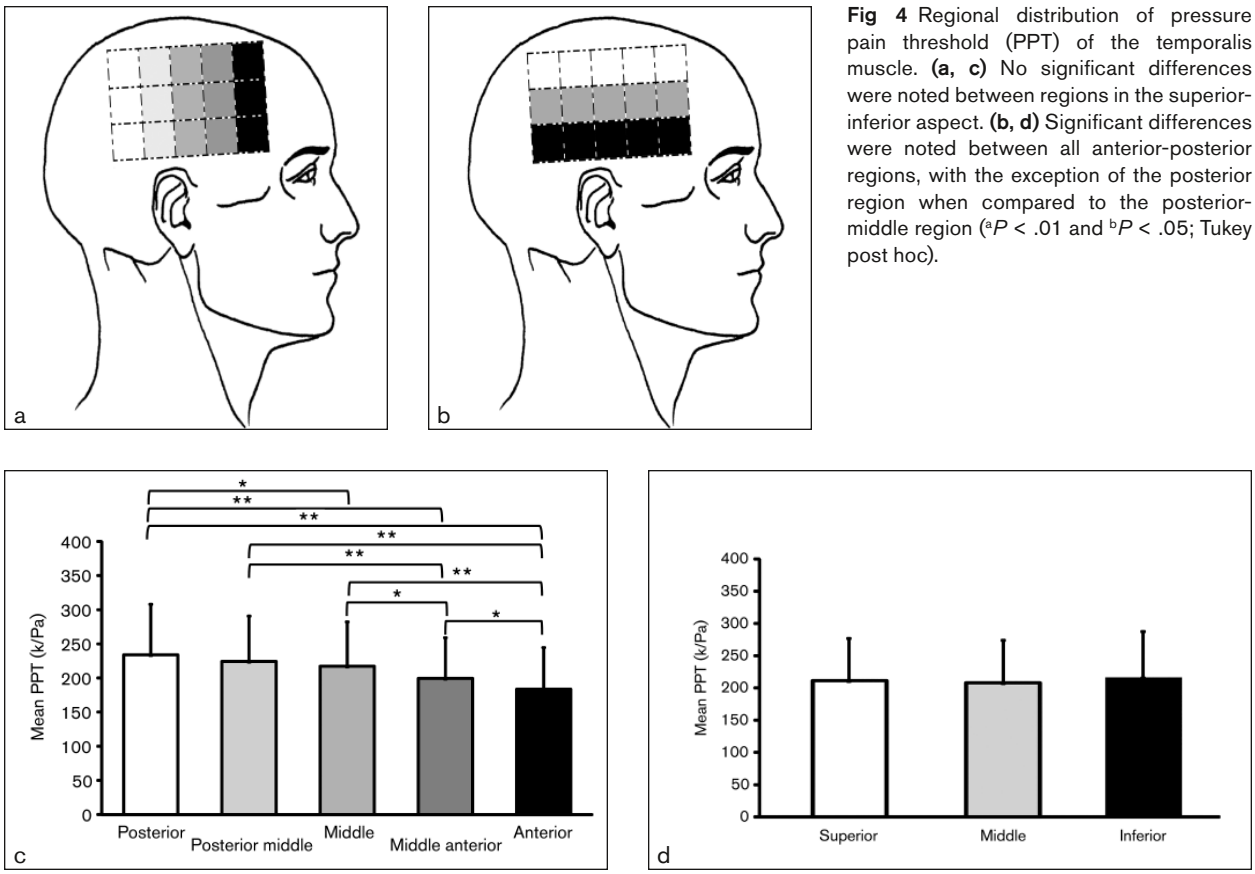

Fig 4 Regional distribution of pressure pain threshold (PPT) of the temporalis muscle. **(a, c)** No significant differences were noted between regions in the superior-inferior aspect. **(b, d)** Significant differences were noted between all anterior-posterior regions, with the exception of the posterior region when compared to the posterior-middle region ($^{a}P < .01$ and $^{b}P < .05$; Tukey post hoc).

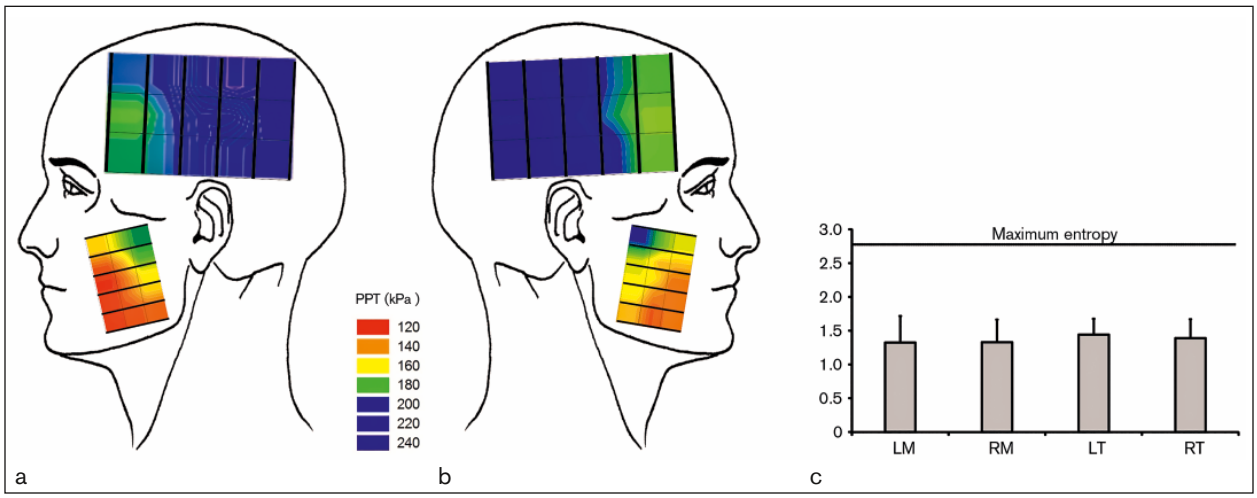

Fig 5 Entropy of pressure pain threshold (PPT) scores. **(a, b)** Graphical representation of the PPT scores obtained in the different points of the temporalis and masseter muscles. Red represents the lowest PPT, and purple represents the highest PPT. **(c)** Mean entropy values for each muscle were evaluated. There were no significant differences for entropy between muscles (masseter vs temporalis) or between sides (right vs left). The maximum entropy is represented as the horizontal line. LM = left masseter; RM = right masseter; LT = left temporalis; RT = right temporalis.

masseter muscle was 1.33 and for the temporalis muscle was 1.42 (Fig 5).

Center of Gravity

There were significant differences in COG for the x coordinate for muscle ($P < .001$; 4-way ANOVA). For the y coordinate, there was a significant interaction of session × side ($F = 12.77$) ($P < .05$; 4-way ANOVA). Also, the COG of the PPT coordinates was plotted for each of the tested muscles in each participant, and showed almost no variation in its physical location (Fig 6).

Age Effects

Pearson correlation showed a moderate but statistically significant correlation between entropy and age ($r = 0.57$, $P < .05$) and between PPT and age ($r = 0.59$, $P < .05$) for the masseter muscle and between entropy and age ($r = 0.65$, $P < .05$) and PPT and age ($r = 0.6$, $P < .05$) for the temporalis muscle (Fig 7).

Discussion

The main findings in this study of healthy individuals were: *(1)* PPTs varied significantly between the masseter and temporalis muscles and also within sites and regions of these muscles, but did not vary between sessions or sides; *(2)* entropy values of PPTs were not different between the masseter and temporalis muscles; *(3)* the COG of the PPTs varied statistically, but these changes do not seem to be clinically relevant for either the masseter or temporalis muscles of healthy subjects; and *(4)* both entropy and PPT showed an overall significant correlation with age for both the masseter and temporalis muscles.

PPT Scores

The present study showed that PPT scores were higher for the temporalis muscle than for the masseter muscle, which is in accordance with most studies,[14,22] but not all.[23] The differences between the muscles could be explained by different morphologic and anatomical characteristics.[6,11,24] In this study, PPT values of the whole of the masseter and temporalis muscles were assessed for the first time. The results showed that there are sites within each muscle that differ significantly from each other in PPT, and results obtained in other studies[14–16] support these variations in PPT. It is important to be aware of this during clinical examination, since palpating the masseter or temporalis muscles with a certain pressure could elicit different pain responses depending on which areas of the muscles are assessed. This, in turn, could lead to the assumption that the area that elicited pain is pathologic when in fact it is a normal variation of the physiology of the muscle. This information supports the use of the concept of familiar pain as described in the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) when patients with TMD are evaluated.[12]

This study reported a mean PPT for the temporalis muscle of 211.7 kPa and for the masseter muscle of 151.0 kPa. Also, no site in either muscle had a mean PPT lower than 130 kPa, which is equivalent to an applied force of 1.32 kg/cm^2. This is of importance,

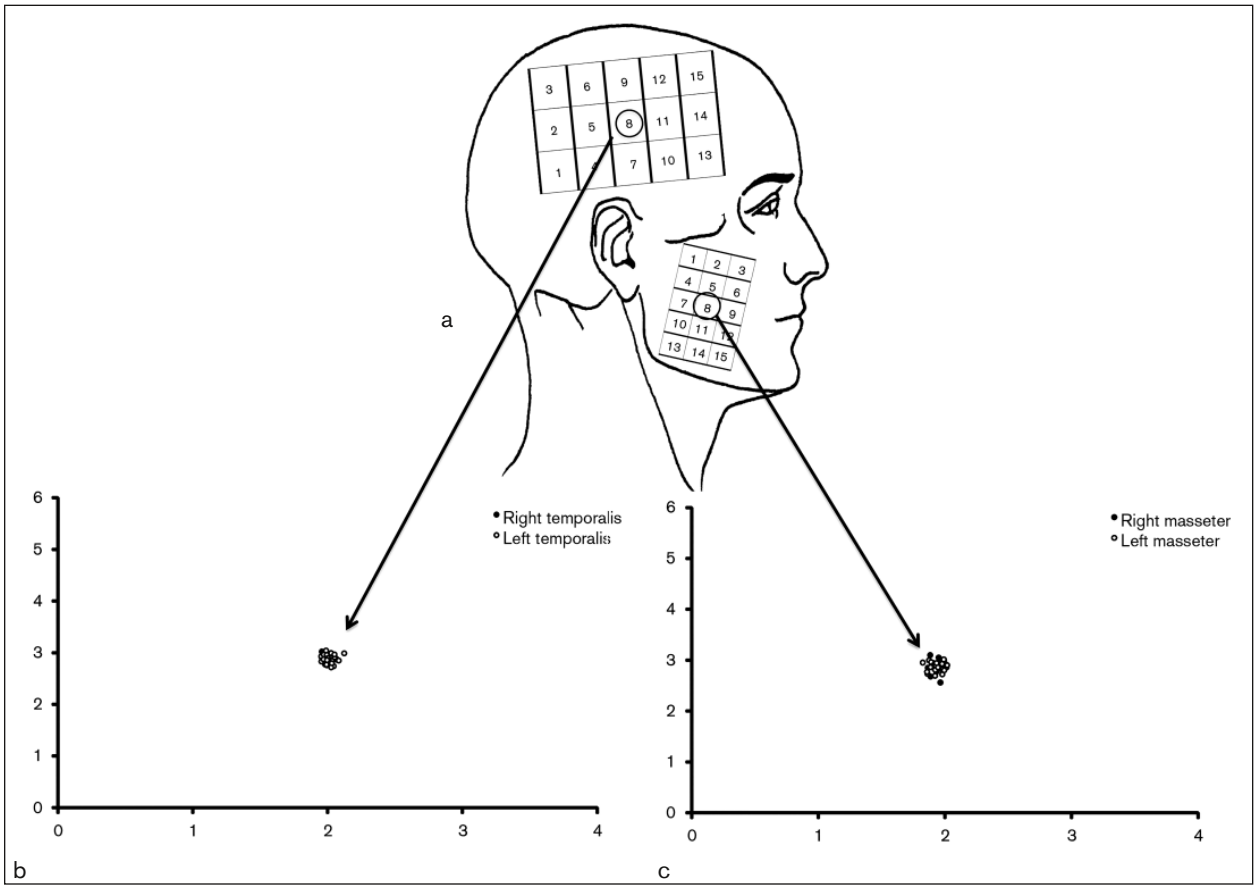

Fig 6 Center of gravity (COG) of pressure pain threshold (PPT). The COG of the PPT was plotted for each participant. **(a)** The assessment grids of the masseter and temporalis muscles are shown overlaying an x/y coordinate system. **(b)** Temporalis and **(c)** masseter muscle COG scores are shown separately on an x/y coordinate system with 3.5y and 2.5y corresponding to the superior and inferior limits, respectively, and 1.5x and 2.5x corresponding to the anterior and posterior limits, respectively, of site 8.

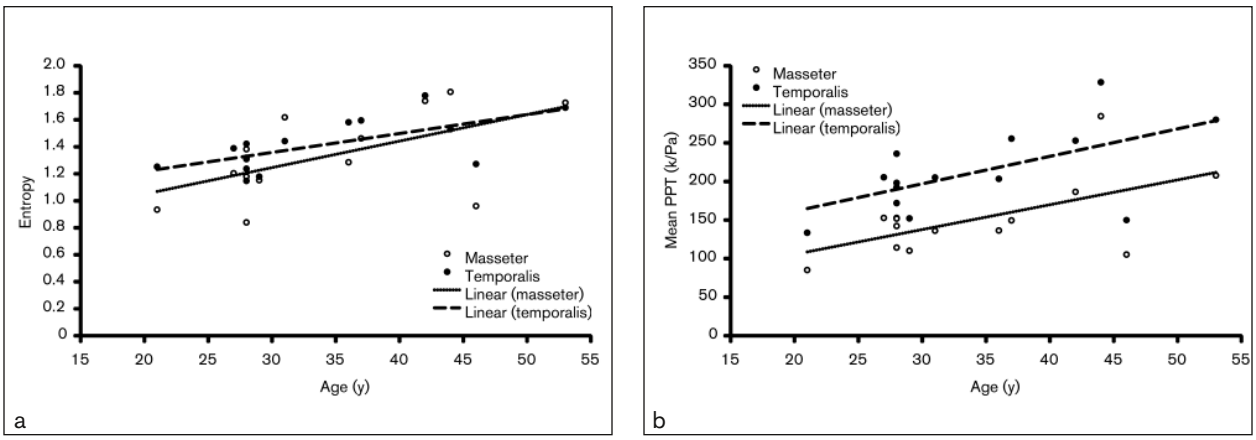

Fig 7 Correlation between age and entropy and between age and pressure pain threshold (PPT). Scatter plots represent Pearson correlation for **(a)** entropy and age **(b)** and PPT and age.

as it is also in accordance with the recommendations from the DC/TMD to not exceed 1.0 kg of force when palpating the masseter and temporalis muscles. These findings emphasize the need for appropriate calibration of the clinician performing the clinical examination and palpation of the orofacial muscles in order to avoid false positive findings that are actually within the normal physiologic range.

The variation of PPT within the muscles was confirmed when dividing the muscles into regions (horizontally and vertically for the masseter muscle and vertically for the temporalis muscle). These variations could be explained by differences in the thickness of the muscles and in the distribution of nerve fibers.[11,14] In this study, a decrease in PPT of the masseter muscle was shown from the superior to the inferior borders and from the posterior to the anterior borders. This can be explained by the innervation of the masseter muscle, since it has been shown that the superior branch of the masseteric nerve innervates mainly the deep layer of the masseter muscle, and that the inferior branch (postero-inferior and antero-inferior) innervates the middle and superficial layers.[25] Furthermore, the richest distribution of the antero-inferior branch of the masseteric nerve is in the areas that, in the present study, were defined as sites 11, 12, 14, and 15, which correspond to the areas with the lowest PPT.

In the temporalis muscle, the PPT decreased from the posterior to the anterior borders. This has been shown to occur only in headache patients and not in healthy controls.[15,16] The differences between these findings and the mentioned studies could be due to differences in the assessed area of the temporalis, as these studies did not assess the whole of the temporalis muscle. The decrease in PPT from posterior to anterior can be explained by the innervation of the muscle, since it has been shown that the innervation density of the anterior region of the temporalis muscle is greater than the posterior region in the number of branches and nerve caliber.[26]

It is interesting to note that for both the masseter and temporalis muscles, the areas of the muscle with the greatest thickness have been shown to be in the anterior or middle region.[26,27] This may indicate that the thicker the muscle, the more innervated it is; and thus the variation in PPT within the muscles may be mainly due to the density and distribution of the nerve fibers and not the muscle thickness. Finally, the present study revealed no gender differences for either of the muscles. There is some controversy about whether PPT differs between genders: Some studies have reported a lower PPT for females than for males,[6,28,29] and others have reported no difference.[30–33] It is interesting to note that the studies that evaluated the PPT of muscles of mastication reported no difference in PPT between genders. Thus, the findings reported here are in accordance with these studies.

Entropy of PPT Scores

The main novelty of this study is the use of the concept of entropy as a way to assess the diversity of PPT values within the masseter and temporalis muscles. Entropy has previously been used to assess physiologic variables such as electromyographic activity[34,35] and also to assess mechanical sensitivity of the masseter muscle.[19] It has recently been proposed that the distribution of pain as a variable should be considered to better characterize the different subgroups of myalgia (ie, local myalgia, myofascial pain, and myofascial pain with referral).[12] Despite this, very little information is known about the spatial distribution of PPTs of the masticatory muscles and if changes in entropy have any relation to pain physiology. In this study, the entropy values of PPT scores in the masseter and temporalis muscles of the healthy subjects were not different between muscles or between sides. The average entropy scores were 1.328 for the masseter muscle and 1.417 for the temporalis muscle, and the maximum entropy score was 2.708. These values are higher than the entropy values (< 1.00) found in a previous study that used palpation of the masseter muscle with a force of 2 kg.[19] This difference could be because the present study used a threshold, whereas the previous study used a specific force applied to the masseter muscle. Considering that this is the first time that the entropy of PPTs was used in this specific way, it is unclear if this is a diverse or nondiverse array of values, but the values found in this study can be proposed as reference values for healthy individuals. A future study evaluating the entropy of PPTs in patients with different diagnoses of myalgia is needed so that a comparison can be made with the values obtained in the present study.

Center of Gravity

The COG of the PPT showed almost no variation in its location, as it was located in the center square of both the masseter and temporalis muscles (square number 8). This indicates that, despite statistical significance of the x and y scores, the COG of the PPT of healthy individuals does not vary significantly between side and muscle. In this sense, the COG measure of mechanical pain sensitivity may be considered a relatively insensitive measure for capturing spatial variation in the masseter and temporalis muscles and could be a disadvantage in diagnostic studies between different groups or in follow-up studies on treatment effects in different muscle pain conditions.

Mechanical Pain Sensitivity and Age

This study found an overall significant correlation between PPT and age and between entropy and age for both the masseter and temporalis muscles; ie, PPT and entropy of both muscles increased with age. Regarding PPT, these results are in accordance with other studies showing an increase in PPT of the muscles of the head and neck with age.[7,29] It is not known if these age changes are due to peripheral or central nervous system changes. Nevertheless, this observation of possible effects of age further strengthens

the suggestion for matched reference groups when differences in mechanical pain sensitivity are tested between different diagnostic groups.[36]

Study Limitations

A limitation of this study may be that only one measurement of PPT for each site in each session was taken. It has been shown that the mean of at least two measurements is better than one measurement alone[14]; however, due to the number of sites that were measured and the need to avoid summation/sensitization, it was decided to take only one measurement per site per session. Another limitation of the study was the relatively small sample size. For this reason, caution is warranted when applying these results to the general population. Studies with larger sample sizes should be undertaken to further describe the within-muscle variability in terms of mechanical pain sensitivity.

Conclusions

This study has shown that it is possible to assess the diversity of PPT values within the masseter and temporalis muscles by using the novel concept of entropy. The results suggest that the anatomical layout of the masseter and temporalis muscles has implications in mechanical pain sensitivity and clearly show that there are areas within these muscles that have lower PPTs than others. Furthermore, reference values for the entropy of PPT scores in healthy individuals have been estimated in this study, and future comparisons of these values with the entropy values of patients with myalgic pain could provide novel information on the spatial heterogeneity of mechanical pain sensitivity in such patients.

Acknowledgments

All authors contributed to and have approved the final version of the manuscript. The authors report no conflicts of interest.

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