

Nerve Growth Factor–Induced Sensitization of the Sternocleidomastoid Muscle and Its Effects on Trigeminal Muscle Sensitivity and Pain Profiles: A Randomized Double-Blind Controlled Study

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Aims: To investigate whether localized sensitization of the sternocleidomastoid (SCM) muscle using nerve growth factor (NGF) would affect masseter and anterior temporalis muscle sensitivity and pain profiles. **Methods:** A total of 28 healthy participants attended two sessions (T_0 and T_1). At T_0 , the maximum voluntary occlusal bite force (MVOBF), as well as pressure pain thresholds (PPT), mechanical sensitivity, and referred pain/sensations for the SCM, masseter, and temporalis muscles, were assessed. Participants also completed the Pain Catastrophizing Scale (PCS), the Pain Vigilance and Awareness Questionnaire (PVAQ), and the Neck Disability Index (NDI). After these assessments, 14 participants received an injection of NGF into the SCM, and 14 received an injection of isotonic saline solution. At T_1 (48 hours postinjection), the participants were again submitted to the same evaluations. **Results:** NGF caused significant mechanical sensitization in the SCM ($P < .025$), but not in the masseter or temporalis muscles ($P > .208$). It also caused significant increases in NDI score ($P = .004$). No statistically significant differences were found for MVOBF, frequency of referred pain/sensations, or questionnaire scores ($P > .248$). **Conclusion:** These results suggest that 48 hours after localized sensitization of the SCM, the primary response is impairment of neck function, but not jaw function. *J Oral Facial Pain Headache 2021;35:7–16. doi: 10.11607/ofph.2593*

Keywords: behavior, masticatory muscles, musculoskeletal pain, neck pain, nerve growth factor

The presence of pain in the cervical muscles and its relation to the functioning of other adjacent cervical and back muscles have been studied using experimental pain models, which have shown that the presence of pain changes the function of agonist and antagonist muscles and is capable of causing hypersensitivity.¹ In the orofacial region, several studies have also used experimental muscle pain models to understand the effect of muscle pain on the functioning of the stomatognathic system.^{2–5} Some of these experimental models are able to modify the muscular behavior that may act as a protective mechanism against further damage and serve to relieve and recover the affected muscle.² One such model is the injection of nerve growth factor (NGF), which can simulate the pain present in myofascial temporomandibular disorders (TMD) and allows a better understanding of the direct effects of a nociceptive stimulus on specific muscle functions.^{5,6} NGF-induced sensitization can be considered a valuable model of muscle pain, since it causes reliable and consistent mechanical sensitization in the short (hours) and long (days) term and does not cause spontaneous pain.⁷ Furthermore, it has been clinically shown that pain in the cervical region can refer to the masticatory muscles.⁸

Since 1950, the craniomandibular system and the cervical spine have been considered a functional biomechanical entity,⁹ and several studies have shown a neurobiologic interaction between head and neck structures.^{10,11} Some authors have demonstrated important trigeminal and cervical connections in the dorsal horn of the upper cervical cord (UCC).¹¹ The trigeminal brainstem sensory nuclear complex is the main region where craniofacial sensory information is processed.¹¹ The UCC is the area of transition between the trigeminal spinal tract subnucle-

us caudalis and the rostral part of the spinal cord.¹⁰ The UCC contains the C1 and C2 segments,¹⁰ while the trigeminal brainstem sensory nuclear complex receives inputs from cranial and cervical nerves and is involved in nociceptive responses to noxious stimuli in the orofacial region.¹¹ Hu et al suggested that these structures might act as one entity to process cutaneous, deep, and visceral nociceptive information from the head and neck areas.¹⁰ The trigemino-cervical-spinal reflex in humans is an example of a reflex interaction between trigeminal afferents and spinal cord neurons, since electrical stimulation of the supraorbital nerve leads to excitatory responses in the neck muscles.¹²

The aim of the present study was to investigate whether localized sensitization of the sternocleidomastoid (SCM) muscle would affect masseter and anterior temporalis muscle sensitivity and pain profiles. The specific hypothesis tested in this study was that sensitization of the SCM would have a detectable impact on daily pain perception, maximum voluntary occlusal bite force (MVOBF), masseter and anterior temporalis muscle sensitivity, and referred pain/sensations.

Materials and Methods

Participants

Healthy individuals were recruited via flyers at the Aarhus University Campus, internet pages of the Section of Orofacial Pain and Jaw Function (<http://odont.au.dk/om-odontologi/sektioner/kof/>), posts on social media, and verbal invitations. Thirty individuals agreed to participate, but 1 was excluded for not meeting the inclusion criteria, and 1 declined to participate in the first appointment. Thus, 28 healthy participants (14 men and 14 women) without signs or symptoms of TMD or neck pain were included (mean age \pm SD: 25.6 \pm 4.2 years; age range: 19 to 37 years; Fig 1). The same examiner (F.P.C.) performed all assessments and experimental procedures at the Department of Dentistry and Oral Health, Aarhus University. The Central Denmark Region Research Ethics Committee approved this study (Opinion: 1-10-72-154-18). All participants received verbal and written information about the study and signed an informed consent form. All procedures were carried out according to the Criteria of Ethics in Research with Humans and in accordance with the Declaration of Helsinki.¹³

The inclusion criteria were healthy individuals, minimum of 18 years old, who had not drunk alcohol in the previous 24 hours, had no more than one missing tooth per hemi-arch and no severe malocclusion, did not report presence of TMD-related pain,¹⁴ and scored a maximum of 5 points (10%) on the Neck

Disability Index (NDI) (no disability or mild disability).¹⁵ The exclusion criteria were individuals who reported regular medication intake, pregnancy, abuse of alcohol or drugs, and/or history of tumor and/or TMD pain.

Study Design

The study was performed in a randomized, double-blind, controlled design. Two sessions (T_0 and T_1) were held 48 hours apart (Fig 1).

In the first session (T_0), the recruited participants answered questions regarding their systemic health, pregnancy, medication intake, drug and alcohol use, and oral health. They also completed the TMD pain screener questionnaire from the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD)¹⁴ and the NDI.¹⁵ After being included and signing the informed consent, participants filled out the Pain Vigilance and Awareness Questionnaire (PVAQ)¹⁶ and the Pain Catastrophizing Scale (PCS).¹⁷ Next, assessments of MVOBF, habitual mastication side (HMS), pressure pain threshold (PPT), mechanical sensitivity, and referred pain/sensations were performed. At the end of the session, participants received an injection of NGF or isotonic saline solution in the SCM. All participants returned 48 hours later for the second session (T_1), where all procedures were repeated, except for the general health-related questionnaire, the TMD pain screener, and the injections.

A program available on the internet (randomization.com) was used to randomize the order of sites tested during the mechanical sensitivity assessments and the injections. A staff member who was not involved in the assessments was responsible for preparing and randomizing the injections in a block design (balanced number of NGF and isotonic saline injections). Both the examiner and participants were blinded to the solution injected (Fig 1). The participants received instructions not to inform the examiner about any sensations and not to take medication or drink alcoholic drinks between the sessions. After injection of all participants, the injected substance was revealed to the examiner, and the participants were divided into the two groups: NGF (experimental, $n = 14$) and saline (control, $n = 14$).

Questionnaires

The PVAQ has 16 questions related to behavior toward pain over the previous 2 weeks.¹⁶ The individuals who complete the questionnaire indicate how frequently each item is a true description of their situation (from 0 = never, to 5 = always).¹⁶ This questionnaire intends to assess responses related to awareness, vigilance, preoccupation, and observation of pain.¹⁶

The PCS has 13 questions related to past painful experiences and aims to assess catastrophic behavior toward pain.¹⁷ The individuals who fill out the ques-

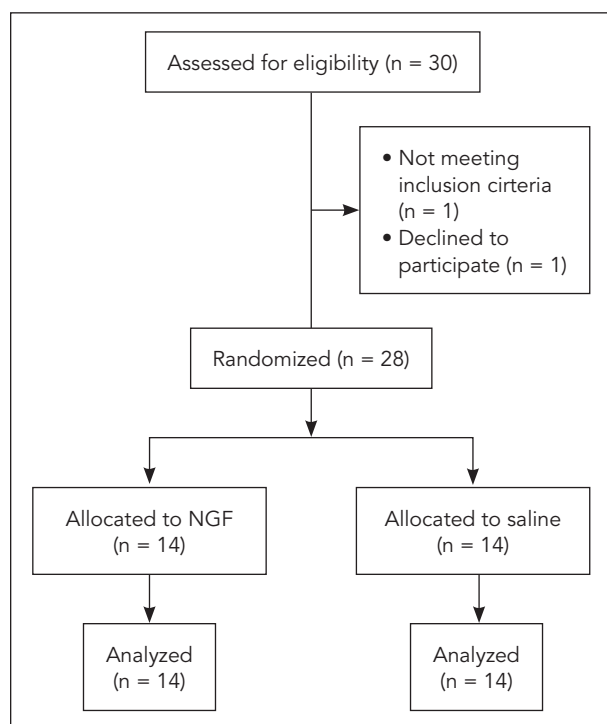


Fig 1 Flowchart of study protocol.

tionnaire give scores based on a 5-point scale, from 0 to 4, where 0 = not at all and 4 = all the time.^{17,18} The questionnaire assesses three subscales: rumination, magnification, and helplessness.^{17,18}

The NDI is divided into 10 sections with 5 options each and aims to assess the effect of neck pain on daily activities.¹⁹ Individuals who fill out the questionnaire choose one score for each item (pain intensity, personal care, lifting, reading, headaches, concentration, work, driving, sleeping, and recreation).¹⁹ The scores for each item vary from 0 to 5 and can be reported as either out of 50 or as a percentage out of 100.¹⁵ The interpretation can be 0 to 4 = none; 5 to 14 = mild; 15 to 24 = moderate; 25 to 34 = severe; and > 34 = complete.¹⁵

MVOBF and HMS

The MVOBF was measured in kilogram force (kgf) with a bite force transducer inserted between the first and second molars and then alternately biting on the right and left sides.²⁰ The participants were instructed to clench with their maximum force for 5 ± 2 seconds²⁰ and then to relax. The assessment was done twice, with 5-minute intervals between the assessments.

The HMS was assessed by visual inspection, as proposed by Kazazoglu et al,²¹ using Stimorol chewing gum (Dandy). The aim of the HMS assessment was to determine the injection side. For the participants who did not have an HMS, the dominant hand determined the injection side.

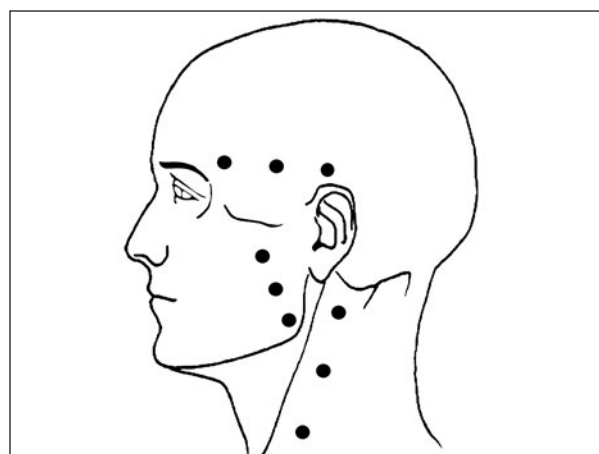


Fig 2 Black dots denoting the mechanical sensitivity assessment sites on each muscle. The original figure can be found in the DC/TMD Assessment Instruments (www.rdc-tmdinternational.org).

PPT and Mechanical Sensitivity Assessments

Before the experiment started, the PPT and mechanical sensitivity instruments were tested on the right flexor muscle of the right thumb to familiarize the participants with the examination, protocol, and scales. PPT and mechanical sensitivity measurements were done on the temporalis, masseter, and SCM muscles on both sides.

An electronic pressure algometer (Somedic SenseLab) with a circular surface of 1 cm² was used to assess PPT. During the assessment, the examiner applied pressure to the muscle site perpendicularly on the skin with a rate of 30 kPa per second, and the participants were requested to press a button when the sensation changed from pressure to pain.²² The muscle sites were assessed three times each, and the average PPT was calculated. The sites on the muscle where the mechanical sensitivity and PPT were evaluated were marked with a red pencil and later recorded using an acetate sheet template (transparency film, 210 × 297 mm, Canon) so the measurement could be repeated on the same site within the session and between sessions.

Simple palpometer devices (Palpeter, Sunstar Suisse) calibrated to 0.5, 1, and 2 kg were used for mechanical sensitivity assessment.²³ The different forces were applied for 2 seconds¹⁴ on three sites of each muscle (anterior, medial, and posterior parts for the temporalis; superior, middle, and inferior parts for the masseter and SCM) (Fig 2). A 0–50–100 numeric rating scale (NRS) was used for the participants to score their sensation at each site, where 0 = no sensation; 1 to 49 = a sensation of pressure but no pain; 50 = barely painful; and 100 = the worst imaginable pain.²⁴ The mean score of the three test sites for each muscle and side was calculated. The participants also had to report if the 2-second pres-

sure stimulus caused referred pain/sensations (yes/no), and, if yes, they drew the area where they felt the referred pain/sensations on a standardized figure.²⁵ Pain/sensations were considered referred if the participant reported them beyond the boundary of the muscle being palpated.²⁶

SCM Injection of NGF or Isotonic Saline

The injections administered were either 0.2 mL of NGF (25 µg/mL in 5-mL vials) or 0.2 mL of isotonic saline solution (0.9%) in the middle of the belly on the upper third of the SCM muscle. This was done by palpating the muscle, and the needle was inserted in an anterior-posterior direction, with the mandible angle as the reference for the region where the needle was inserted. Aspiration test was done before slow injection (about 10 seconds) of the 0.2-mL volume into the muscle tissue.

Statistical Analyses

Statistica software (version 10, StatSoft) was used for statistical analysis. Data were assessed for normality using quantile-quantile (Q-Q) plots, and all data were shown to be normally distributed.

For all questionnaires and assessments, the data were analyzed using repeated-measures analysis of variance (ANOVA), with gender (man/woman) and substance (NGF/saline) as the independent variables, and NDI, PCS, PVAQ, MVOBF, PPT, and mechanical sensitivity as the dependent variables. The data were analyzed as follows:

- NDI and PVAQ data with session (T_0/T_1) as a within-subject factor.
- PCS data were analyzed for PCS total score and PCS subscales, with session (T_0/T_1) as a within-subject factor.
- MVOBF with side (injection/noninjection) and session (T_0/T_1) as within-subject factors.
- PPT with side (injection/noninjection), muscle (temporalis/masseter/SCM), and session (T_0/T_1) as within-subject factors.
- Mechanical sensitivity data were analyzed separately for each muscle. Session (T_0/T_1), force (0.5/1/2 kg), side (injection/noninjection), and area (anterior/medial/posterior for temporalis; upper/middle/lower for masseter and SCM) were considered as within-subject factors.

Tukey honest significant difference test was used for post hoc comparisons. The data obtained from referred pain/sensation reports were assessed with McNemar exact two-tailed test. Muscles were compared in pairs (temporalis × masseter; temporalis × SCM; masseter × SCM), and between T_0 and T_1 for each muscle. $P < .05$ was considered significant.

A sample size estimate suggested that with 10 participants in each group ($N = 20$), a 20% (SD 16%) difference in mean mechanical sensitivity could be detected between groups, with $\alpha = .05$ and $\beta = 0.2$, yielding a power of 80%.

Results

All 28 participants completed the two sessions, with no dropouts, missing data, or any side effects.

Questionnaires

Three-way ANOVA demonstrated no significant effect of gender ($F = 0.603$; $P = .804$), session ($F = 1.906$; $P = .174$), or substance ($F = 1.719$; $P = .202$), and no significant interaction for session × substance, for the PVAQ ($F = 2.371$; $P = .137$). For the PCS total score, there was no significant difference for any of the analyzed factors or for the PCS subscales. Four-way ANOVA demonstrated a significant effect of subscale ($F = 23.19$; $P < .001$), but not of gender ($F = 0.40$; $P = .535$), session ($F = 0.0$; $P = 0.958$), or substance ($F = 0.36$; $P = 0.555$), and the interaction subscale × session × substance ($F = 1.33$; $P = .275$) was not significant. Three-way ANOVA for the NDI demonstrated no significant effect of gender ($F = 0.04$; $P = .836$), but significant effects of session ($F = 11.91$; $P = .002$) and of substance ($F = 5.71$; $P = .025$), as well as a significant interaction for session × substance ($F = 9.93$; $P = .004$). The NDI scores were calculated in percentages. Table 1 shows the PVAQ, PCS, and NDI scores at T_0 and T_1 , with significantly higher scores at T_1 following NGF injection compared to isotonic saline for the NDI. No statistically significant difference was found between groups at T_0 for any of the questionnaires.

MVOBF and HMS

Four-way ANOVA for MVOBF demonstrated that there was no significant effect of gender ($F = 1.010$; $P = .325$), side ($F = 0.384$; $P = .541$), session ($F = 0.0$; $P = .696$), or substance ($F = 3.0$; $P = .079$), nor a significant interaction among the factors (gender × side × session × substance; $F = 0.384$; $P = .541$). The average MVOBF for NGF at T_0/T_1 was 41.9/44.8 kgf, respectively, on the injection side and 44.7/46.8 kgf on the noninjection side. For the saline group at T_0/T_1 , these values were 59.6/57.5 kgf on the injection side and 58.8/58.5 kgf on the noninjection side. No statistically significant difference was found between groups at T_0 for any of the assessments.

The HMS test showed that 11 participants had the right side as their HMS, 10 their left side, and 7 could not define. For these 7 cases, the right side was used for injection.

Table 1 Mean (SD) Scores for the NDI, PVAQ, and PCS at T₀ and T₁

| Questionnaire | Injection | Session | | P |
|---------------|-----------|------------------------|-------------------------|-------------------|
| | | T ₀ | T ₁ | |
| NDI | NGF | 3.3 (3.9) ^a | 12.7 (9.0) ^a | .004 ^b |
| | Saline | 3.7 (3.4) | 4.1 (4.6) | |
| PVAQ | NGF | 41.0 (14.9) | 41.3 (11.8) | .137 |
| | Saline | 37.4 (13.43) | 31.4 (15.9) | |
| PCS | NGF | 15.6 (6.7) | 15.5 (10.8) | .959 |
| | Saline | 13.3 (8.6) | 13.0 (11.5) | |

NDI = Neck Disability Index; PVAQ = Pain Vigilance and Awareness Questionnaire; PCS = Pain Catastrophizing Scale.

^aStatistically significant ($P < .05$) difference between T₀ and T₁.

^bStatistically significant interaction for session × substance.

Table 2 Mean (SD) Pressure Pain Threshold in NGF and Saline Groups at T₀ and T₁

| Substance | Injection side | | | | | | Noninjection side | | | | | |
|-----------|----------------|----------------|----------------|----------------|---------------------|---------------------|-------------------|----------------|----------------|----------------|----------------|----------------|
| | Temporalis | | Masseter | | SCM | | Temporalis | | Masseter | | SCM | |
| | T ₀ | T ₁ | T ₀ | T ₁ | T ₀ | T ₁ | T ₀ | T ₁ | T ₀ | T ₁ | T ₀ | T ₁ |
| NGF | 189.3 | 205.4 | 174.0 | 181.6 | 112.0 | 90.5 | 190.8 | 190.0 | 187.6 | 181.8 | 101.6 | 102.6 |
| | (61.4) | (68.7) | (44.4) | (66.0) | (37.7) ^a | (43.1) ^a | (60.0) | (69.0) | (57.59) | (71.69) | (35.4) | (32.5) |
| Saline | 197.9 | 206.8 | 195.4 | 201.2 | 128.7 | 133.0 | 194.1 | 212.7 | 186.8 | 203.1 | 127.5 | 136.2 |
| | (62.4) | (55.0) | (60.8) | (62.5) | (49.0) | (45.4) | (69.1) | (66.0) | (57.0) | (65.2) | (48.8) | (49.0) |

^aStatistically significant difference ($P < .05$) between T₀ and T₁.

PPT and Mechanical Sensitivity

For PPT measurements, five-way ANOVA demonstrated a significant effect of muscle ($F = 153.9$; $P < .001$), but not gender ($F = 1.9$; $P = .179$), side ($F = 0.0$; $P = .973$), session ($F = 0.02$; $P = .167$), or substance ($F = 0.9$; $P = .362$). There were significant interactions among the factors side × muscle × session × substance ($F = 6.1$; $P = .004$). Tukey test showed significantly lower PPTs at the SCM compared to the masseter and temporalis at T₀ and T₁ for both the saline and NGF groups, but only for the SCM could the post hoc test identify a significant decrease in PPT at T₁ compared to T₀ on the injected side (Table 2).

Regarding mechanical sensitivity, the six-way ANOVA for the temporalis demonstrated significant effect of force ($F = 100.5$; $P = .000$) and area ($F = 24.4$; $P = .000$), but not side ($F = 1$; $P = .319$), gender ($F = 0.6$; $P = .431$), session ($F = 0.4$; $P = .546$) or substance ($F = 0.0$; $P = .89$). There were no significant interactions among the factors force × side × session × substance ($F = 1.8$; $P = .183$), nor among the factors side × area × session × substance ($F = 0.16$; $P = .855$).

The six-way ANOVA for the masseter demonstrated a significant effect of force ($F = 168.6$; $P = .000$)

and area ($F = 4.4$; $P = .018$), but not gender ($F = 2.2$; $P = .148$), side ($F = 1$; $P = .326$), session ($F = 0.8$; $P = 0.376$), or substance ($F = 0$; $P = .848$). There were no significant interactions among the factors force × side × session × substance ($F = 0.1$; $P = .941$), nor among the factors side × area × session × substance ($F = 1.5$; $P = 0.243$).

Six-way ANOVA for the SCM demonstrated a significant effect of force ($F = 242.7$; $P = .000$), side ($F = 27.6$; $P < .001$), and area ($F = 9.9$; $P < .001$), but not gender ($F = 1.2$; $P = .278$), session ($F = 4.3$; $P = .050$), or substance ($F = 1.7$; $P = .208$). There were significant interactions among the factors force × side × session × substance ($F = 4$; $P = .025$) and among the factors area × side × session × substance ($F = 12.2$; $P < .001$). When considering the interaction force × side × session × substance, Tukey test for SCM showed that, in the NGF group, on the injection side, there was a statistically significant increase of mechanical sensitivity scores for all forces. There was no significant difference between T₀ and T₁ on the noninjection side. As expected, the higher NRS scores were observed for 2 kg on both sides and both sessions. For the saline group, there was a statistically significant difference between the forces (the higher the force, the higher the scores),

Table 3 Mean (SD) Mechanical Sensitivity Scores in the Temporalis, Masseter, and Sternocleidomastoid (SCM) for Different Forces and Injection Sides in NGF and Saline Groups at T₀ and T₁

| Force | | 0.5 kg | | | | 1 kg | | | | 2 kg | | | |
|----------------|--------|-----------------------------|-----------------------------|----------------|----------------|------------------------------|-----------------------------|----------------|----------------|-----------------------------|-----------------------------|----------------|----------------|
| Injection side | | Injection | | Noninjection | | Injection | | Noninjection | | Injection | | Noninjection | |
| Session | | T ₀ | T ₁ | T ₀ | T ₁ | T ₀ | T ₁ | T ₀ | T ₁ | T ₀ | T ₁ | T ₀ | T ₁ |
| Temporalis | NGF | 6.3 (5.1) | 5.7 (5.3) | 6.3 (6.6) | 5.5 (6.3) | 13.7 (10.2) | 11.6 (6.8) | 13.0 (9.8) | 10.9 (6.4) | 22.0 (11.9) | 18.6 (11.9) | 23.2 (14.5) | 22.6 (1.8) |
| | Saline | 6.7 (9.9) | 5.9 (8.3) | 4.3 (4.2) | 4.9 (4.4) | 11.4 (11.4) | 12.4 (13.4) | 8.0 (6.9) | 10.0 (8.7) | 25.6 (16.9) | 26.5 (13.4) | 24.5 (13.2) | 23.9 (14.7) |
| Masseter | NGF | 10.2 (6.6) | 8.8 (6.8) | 10.2 (7.8) | 7.8 (9.4) | 20.1 (11.4) | 19.5 (12.9) | 19.6 (12.5) | 15.3 (8.9) | 36.9 (16.1) | 34.9 (17.1) | 39.7 (16.8) | 34.4 (16.8) |
| | Saline | 9.5 (12.1) | 7.8 (10.3) | 9.9 (12.0) | 7.7 (9.5) | 17.7 (16.4) | 20.8 (16.6) | 20.0 (18.4) | 18.9 (16.3) | 38.0 (17.6) | 41.9 (16.1) | 37.5 (17.5) | 37.3 (15.0) |
| SCM | NGF | 13.3 (10.7) ^a | 27.5 (20.9) ^a | 12.6 (9.5) | 10.5 (8.2) | 27.33 (13.9) ^a | 42.6 (20.1) ^a | 25.1 (13.4) | 23.3 (13.5) | 50.3 (16.6) ^a | 58.8 (17.8) ^a | 49.8 (17.9) | 48.3 (18.8) |
| | Saline | 9.5 (10.4) | 11.9 (13.9) | 9.4 (12.1) | 11.1 (13.5) | 23.3 (17.4) | 25.2 (15.4) | 21.4 (16.1) | 23.1 (15.9) | 47.1 (17.9) | 51.1 (13.2) | 47.5 (16.3) | 44.7 (15.7) |

^aStatistically significant difference ($P < .05$) between T₀ and T₁.

Table 4 Participants in Each Group Reporting Referred Pain/Sensations for the Different Muscles and Sessions

| | Temporalis | | | | | | Masseter | | | | | | Sternocleidomastoid | | | | | | |
|--------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|---------------------|----------------|----------------|----------------|----------------|----------------|---|
| | 0.5 kg | | 1 kg | | 2 kg | | 0.5 kg | | 1 kg | | 2 kg | | 0.5 kg | | 1 kg | | 2 kg | | |
| | T ₀ | T ₁ | T ₀ | T ₁ | T ₀ | T ₁ | T ₀ | T ₁ | T ₀ | T ₁ | T ₀ | T ₁ | T ₀ | T ₁ | T ₀ | T ₁ | T ₀ | T ₁ | |
| NGF | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Saline | 0 | 0 | 1 | 2 | 2 | 1 | 1 | 0 | 0 | 1 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |

N > 9 because some participants reported referred pain when different muscles were palpated.

but no difference was found between T₀ and T₁ for any of the applied forces (Table 3). When considering the interaction area × side × session × substance, Tukey test for SCM showed that, in the NGF group, on the injection side, the upper and middle areas had a statistical difference and were more sensitive to mechanical pressure after the NGF injection. On the other hand, the lower SCM area did not have significant changes in the NRS scores. There was no statistically significant difference on the noninjection side. For the saline group, there was no statistical difference between T₀ and T₁ for any area or side. No statistically significant difference was found between groups at T₀ for any of the assessments.

Thus, sensitization with NGF did not affect the mechanical sensitivity of the temporalis or masseter, and the sensitization was concentrated around the injection area in the SCM.

Referred Pain/Sensations

Nine participants (32%; two men and seven women; four NGF and five saline) reported referred pain/sensations during the mechanical sensitivity assessment. McNemar exact two-tailed test showed no statistically significant difference between the muscles at T₀ (temporalis × masseter: $P = .6831$; temporalis × SCM: $P = .4795$; masseter × SCM: $P = .2482$) or at T₁ (temporalis × masseter: $P = .4795$; temporalis × SCM: $P = .6171$; masseter × SCM: $P = 1.000$). There was no statistically significant difference between T₀ and T₁ for the analysis of each muscle (temporalis: $P = .4795$; masseter: $P = .6171$; SCM: $P = .4795$) (Table 4).

The muscle with the highest frequency of referred pain/sensations was the masseter muscle (9 times in 5 different individuals). When considering the force, the 2-kg stimulus had more referred pain/sensation reports (10 times in 8 different participants). Although

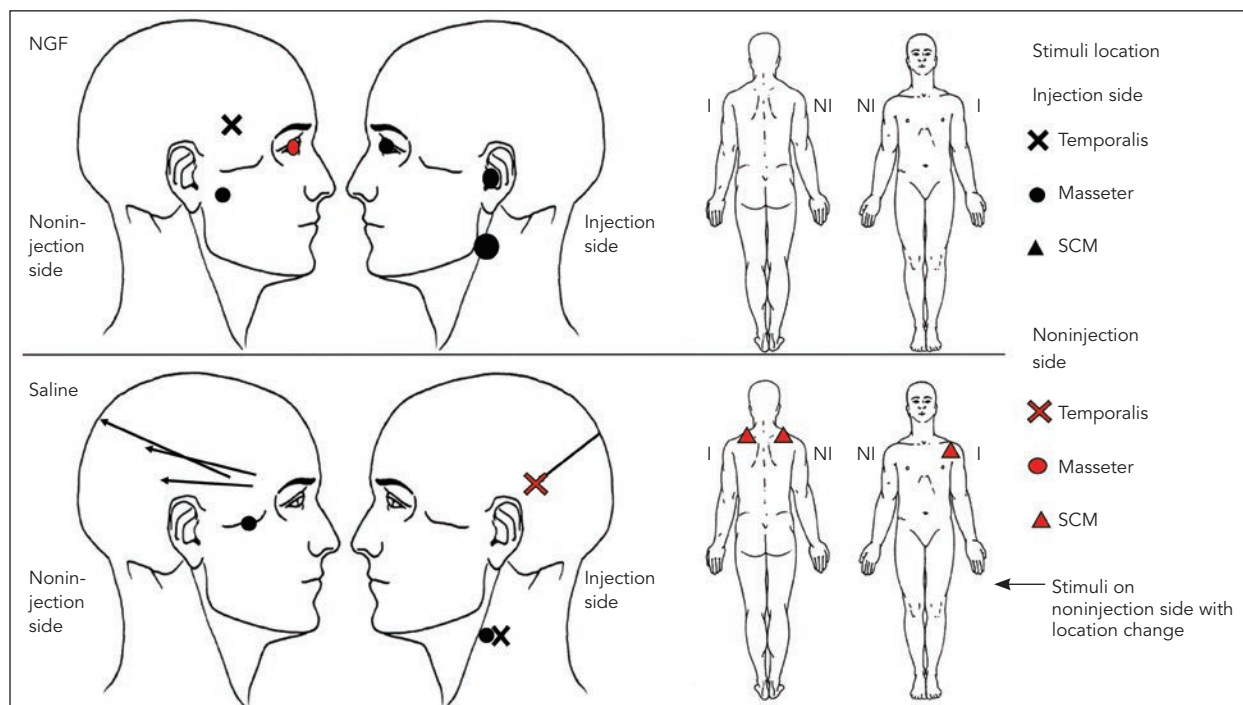


Fig 3 Areas where the participants drew their referred pain/sensations during mechanical sensitivity tests. The symbols represent the stimulus location and are placed on the figures according to where the participants drew the referred pain/sensation. The original figures can be found in the DC/TMD Assessment Instruments, www.rdc-tmdinternational.org.

the SCM presented mechanical sensitization, there were only 2 reports of referred pain/sensations in the saline group when this muscle was palpated. The areas where the participants drew the referred pain/sensations varied in several areas of the head, neck, and shoulders (Fig 3).

The present study clearly demonstrated local sensitization of the SCM muscle associated with impairment in the NDI, a lack of change in pain catastrophizing scores, a conspicuous lack of sensitization of the trigeminal muscles and referred pain/sensations, and no overall effects on MVOBF of the jaw muscles.

Discussion

This study showed that an injection of NGF caused a clear-cut sensitization of the SCM, as shown by the NDI, PPT, and mechanical sensitivity scores, but without any effect on the MVOBF, PPT and mechanical sensitivity, or referred pain/sensations of the temporalis and masseter muscles. Consequently, the overall tested hypothesis was rejected. The choice of NGF as substance to cause experimental pain in this study was due to the fact that NGF is able to mimic characteristics of myofascial pain, such as pain during muscle palpation and function.^{6,28,29} In addition, NGF causes longer-lasting pain in com-

parison with glutamate.⁴ NGF is a protein present in the human organism that participates in the peripheral sensitivity of the nociceptive system. Its level is increased in several inflammatory processes,³⁰ and when injected in healthy muscles, it is capable of causing thermal and mechanical hyperalgesia.^{4,31} Svensson et al suggested that peripheral and central mechanisms could be responsible for mechanical hyperalgesia caused by this protein injection.⁴ Thus, the use of NGF has been a valuable tool in studies involving experimental muscle pain.^{4,5,29} According to the present authors' knowledge, this is the first study to test the effects of NGF injection into the SCM on daily pain perception, MVOBF, and sensory changes in the temporalis, masseter, and SCM muscles.

Studies have associated the presence of pain catastrophizing and pain hypervigilance with chronic musculoskeletal pain.^{32,33} Other studies that evaluated experimental^{34,35} and acute pain episodes³⁶ have shown that pain awareness and catastrophizing are associated with higher pain perception. However, it is difficult to determine if catastrophizing behavior causes worse pain adjustment or vice versa.³⁷ This study showed that an NGF injection in the SCM did not have an influence on pain hypervigilance or catastrophizing, as assessed by questionnaires. This is most likely because the assessments in this study were done at 48 hours postinjection, which is a short

time period. Individuals with longer pain duration (3 months or more) have higher hypervigilance scores than individuals with acute pain or recurrent pain.³⁸ Sampaio Bonafé et al explained that individuals with these types of pain present similar scores for hypervigilance, and that those might be considered as isolated events not influencing hypervigilance.³⁸ It is possible to infer that experimental pain, as in the present study, has similar effects to acute and recurrent pain. On the other hand, the NDI showed significant increases at T₁ in the group that received the NGF injection. The NDI assesses how neck pain affects the ability to manage in everyday life; the higher the score, the higher the impact of neck pain on daily activities.¹⁵ Thus, as expected, sensitization of the SCM via NGF injections affected the participant's daily activities, as assessed by the NDI.

Previous research has shown that pain in the masseter muscle is related to a lower bite force in the molar region^{39,40}; however, this study showed that when the sensitization occurs in the SCM, there is no change in MVOBF. Maybe in patients with chronic neck pain it would be possible to find decreases in MVOBF, as explained by the integrated pain adaptation model.⁴¹ However, the MVOBF recordings were not associated with spontaneous pain reports from the masseter or temporalis muscles, and no indication of sensitization of these muscles was seen. This is consistent with the notion, from the integrated pain adaptation model, that (jaw) motor function may not change if muscle contraction or movements do not lead to increased levels of pain.^{40,42} Further studies with longer follow-up and with participation of patients with chronic pain in the SCM could further test this hypothesis.

Similarly to the study of Schmidt-Hansen et al,²⁷ the SCM PPT was lower than for the temporalis and masseter muscles. In contrast to the initial hypothesis, SCM hyperalgesia did not change temporalis and masseter responses to mechanical stimuli (ie, PPT and mechanical sensitivity). This hypothesis was formulated due to the evident biomechanical and neurologic interactions between head and neck structures⁹⁻¹¹ and the trigeminal-cervical reflexes.¹² In the present study, the assessments were made within a 48-hour interval; the responses to head and neck stimuli were not a two-way path. On the other hand, Testa et al suggested that in patients with chronic neck pain, there is a bidirectional relationship between jaw/neck pain and motor disturbances.⁴³ Thus, further studies with different levels of painful stimuli and longer-lasting sensitization could investigate the mechanical and neurophysiologic responses to stimuli applied to the SCM and other neck muscles.

Muñoz-García et al assessed psychosocial aspects and neck disability in patients with cervical-

gia with and without TMD, as well as asymptomatic patients, and compared the pain thresholds of the anterior temporalis and masseter muscles to nontrigeminal muscles (trapezius and tibialis).⁴⁴ Their results showed that the PPTs of all evaluated muscles were lower in the group presenting TMD and neck pain.⁴⁴ The present participants were asymptomatic for TMD, and T₁ happened 48 hours after the injections. The short period with sensitization might be another explanation for the absence of significant changes in the response of the temporalis and masseter muscles. Perhaps a longer period of SCM hyperalgesia would be capable of causing significant changes in PPT and mechanical sensitization of the masseter and temporalis muscles. In the present study, NGF injection was efficient in causing local sensitization of the SCM, leading to the decrease of PPT and increases in mechanical sensitivity. However, it seems that the sensitization was mainly local, and it did not spread along the SCM nor lead to detectable sensitization of the masticatory muscles.

Regarding referred pain/sensations, the sensitization of the SCM did not lead to significant differences between T₀ and T₁ for any of the evaluated muscles. The number of participants who reported referred pain/sensations was very similar, with one more individual in the saline group. This reinforces the idea that sensitization of the SCM with NGF was not capable of influencing referred pain/sensations. The majority of the referred pain/sensations were most commonly evoked with the 2-kg stimulus, and it was not dependent on test site. The present finding corroborates the study of Schmidt-Hansen et al, where pain drawings showed referred pain/sensations in the trigeminal and cervical innervation territories.²⁷ Although researchers suggest an overlap of the trigeminal and cervical afferent projections,⁴¹ the present study did not find referred pain/sensations in the facial region during palpation of the SCM after the NGF injection. It is worth mentioning that finding and palpating hyperexcitable areas of the temporalis, masseter, and SCM was not the aim of the present study. Furthermore, in this study, the palpation lasted for 2 seconds instead of the 5 seconds recommended by the DC/TMD.¹⁴ This could explain the relatively low prevalence of referred pain/sensations. Masuda et al found that mechanical stimuli for 2 seconds are capable of evoking referred pain/sensations, but that the frequency of responses is higher with 5 and 10 seconds.²⁶

Wong et al demonstrated that NGF injection in the masseter muscle of rats increased expression of NMDA receptor subtype 2B in neurons of the trigeminal masseter ganglion, which peaked at 3 days postinjection and verified an increase of expression of calcitonin gene-related peptide and substance P in rats.²⁹ It is likely that the NGF injection in the

SCM caused the increase of these substances only in SCM neurons and not in the masseter or temporalis.

Conclusions

Although the SCM is often assumed to be linked to painful TMD, the present study suggests that 48 hours after localized sensitization of the SCM, the primary response is impairment of neck function, but not jaw function. These findings may have clinical implications for the understanding of the complex interactions between the cervical and trigeminal systems.

Clinical Implications

- According to the authors' knowledge, this study was the first to test the effect of an NGF injection pain model in the human SCM muscle.
- The injection into the SCM showed impairment of neck function and local sensitization. However, no effect on trigeminal muscles or pain profiles was observed within the 48 hours.
- The studied pain model did not seem to be ideal to mimic the effect of myofascial pain in the neck within the study frame.

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