Comparison of Pain-Generated Functional Outcomes in Experimental Models of Delayed-Onset Muscle Soreness and Nerve Growth Factor Injection of the Masticatory Muscles

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Submitted November 2, 2019;
accepted April 8, 2020.

Aims: To compare two pain models of myalgic TMD, delayed-onset muscle soreness (DOMS) and injections of nerve growth factor (NGF), in terms of pain-related and motor function outcomes, as well as activity-related temporal summation. **Methods:** Fifty age- and gender-matched healthy participants were recruited and randomized into one of three groups: to a repeated eccentric contraction task to cause DOMS (n = 20), to receive NGF injections into the masseter muscle (n = 20), or to a control group (n = 10). Mechanical sensitivity of masticatory muscles, chewing parameters, jaw function limitation, maximum bite force, and activity-related temporal summation were assessed at baseline and at days 1, 2, and 7 following the intervention. **Results:** Compared to baseline, both model groups showed increased mechanical sensitivity, jaw function limitation, pain on chewing, and decreased chewing efficiency, lasting longer in the NGF group than in the DOMS group ($P < .05$). Furthermore, also compared to baseline, the NGF group showed increased pain on maximum bite and decreased pain-free maximum opening ($P < .05$). No increases in activity-related temporal summation were shown for any of the model groups when compared to baseline or the control group ($P > .05$). **Conclusion:** Both models produced similar pain-related outcomes, with the NGF model having a longer effect. Furthermore, the NGF model showed a more substantial effect on motor function, which was not seen for the DOMS model. Finally, neither of the models were able to provoke activity-related temporal summation of pain. *J Oral Facial Pain Headache 2020;34:311–322. doi: 10.11607/ofph.2623*

Keywords: *experimental pain, mechanical sensitivity, myalgia, temporal summation, trigeminal motor physiology*

It has been shown that painful temporomandibular disorders (TMD) can alter jaw motor function, leading to, for example, slower velocity during mouth opening or decreased range of motion.[1–3] The influence of myalgia on jaw motor function has been previously explained by the pain adaptation model, in which the motor system is set up to limit movements and provide a chance for recovery of the masticatory system when pain is present.[3,4] Later, it was updated to account for other aspects of pain (ie, biologic and psychosocial), providing a better understanding of the way pain affects motor function in the orofacial region.[5]

Experimental pain models, such as glutamate or hypertonic saline injections into the jaw muscles, resisted jaw movements, and intense dynamic prolonged chewing, have been used to elucidate how pain may affect motor function of the masticatory muscles.[6–10] However, due to the short-lasting effect of these models, they do not seem to have a substantial effect on motor function.[11,12] For example, pain caused by hypertonic saline has been shown to have no effect on kinematic parameters of chewing.[13,14] Additionally, it has been shown that jaw movements initiated after a hypertonic saline injection relieve the pain rather than increase it, unlike in patients with painful TMD.[15]

Recently, two experimental pain models, the effects of which last up to a few days, have been used to assess the effects of pain in a variety of situations.[16–20] Nerve growth factor (NGF) has been shown to be a major peripheral factor in the development of myalgia.[21] Furthermore, intramuscular injections of NGF reproducibly cause dose-dependent allodynia

and hyperalgesia for at least 7 days.[22–25] In addition, a model of delayed-onset muscle soreness (DOMS) for the masticatory muscles has been developed,[16,26] based on a combination of eccentric and concentric contractions of the masticatory muscles. This model causes the development of pain by lengthening the masticatory muscles repeatedly while contracting. The provoked pain occurs several hours after the exercise and peaks between 24 and 48 hours.[16,27] Both models cause symptoms similar to those in patients with myalgic TMD, such as pain on palpation and jaw movement, reduced voluntary muscle force, and limited pain-free range of movement.[19,20,26,28,29]

It has been shown previously that temporal summation of pain caused by repeated motor activity is enhanced in chronic pain conditions.[30,31] Furthermore, activity-related temporal summation of pain was successfully induced in TMD pain patients by repeated mandibular movements.[32] However, the possibility of activity-related temporal summation of pain occurring as a consequence of an experimental pain model of the masticatory muscles has yet to be investigated.

The aim of this study was to compare the efficacy of two myalgic TMD models—NGF and DOMS—in regard to pain-related outcomes, motor function outcomes, and activity-related summation of pain. The hypothesis was that NGF-induced changes would be more prominent and last longer than those caused by the DOMS model.

Materials and Methods

Participants

The sample size was calculated by setting risks for type I and type II errors of 5% and 20%, respectively. Mean and variance values of experienced pain were obtained from previous studies.[16–20,33–35] The size of the control group was set as half that of the DOMS and NGF groups. The calculation showed a total of 45 patients should be included. To account for drop-outs, 50 healthy participants (30 men and 20 women) were enrolled, and stratified random sampling was used for gender and age matching. The participants were recruited via advertisements in flyers mounted inside the Aarhus University Campus and posted on web pages of the Section of Orofacial Pain and Jaw Function.

The inclusion criteria were as follows: participants should be at least 18 years old, with no systemic disorders (eg, rheumatologic diseases, heart diseases, psychologic disorders) and no missing teeth except for the third molars. Exclusion criteria were: history of neuromuscular diseases; musculoskeletal injuries; chronic orofacial pain complaints, headaches, or any TMD-related symptoms according to the Diagnostic Criteria for TMD (DC/TMD)[36]; prolonged use of medication (with the exception of birth control pills); and/or use of painkillers during the trial or 24 hours before the start of the trial.

Twenty of the participants (mean ± SD age 30 ± 11 years) received repeated mechanical provocation to cause DOMS in the jaw muscles, another 20 participants (27 ± 10 years) received injections of NGF into the masseter muscle on both sides, and 10 participants (29 ± 11 years) had only the assessments performed, serving as the control group. All participants were notified of the procedures and purpose of the study, and informed written consent was obtained from all volunteers before initiation of the study. The study was approved by the Central Denmark Region Research Ethics Committee (1-10-72-154-18) and conducted in accordance with the Declaration of Helsinki.

Study Protocol

Assessments were done at baseline (T_0) and 1, 2, and 7 days after the baseline session (T_1, T_2, and T_7, respectively). Assessments on each day consisted of an assessment for TMD according to the DC/TMD[36]; completion of the Oral Health Impact Profile (OHIP),[37] Jaw Functional Limitation Scale (JFLS),[38] and Oral Behavior Checklist (OBC) questionnaires[39]; mechanical sensitivity and referred sensation (RS) assessments; and pain intensity recordings during maximum bite, chewing, and repeated open-close movements. Furthermore, the assessment of motor function included the recording of maximum opening (with pain and without pain), maximum bite force (MBF), chewing efficiency, and repeated-opening measurements. In addition, the participants were asked to rate pain, unpleasantness, tiredness, tension, soreness, and stiffness of the muscles in the morning and afternoon every day of enrollment in the study.[40,41] Participants were randomly assigned, and the order in which the forces were applied and the sites were assessed was also randomized.

DOMS Provocation

DOMS was provoked according to a previously described protocol.[26] An apparatus was used to stretch the jaw-closing muscles of the participants when they were clenching at 10% of maximum force in order to perform eccentric and concentric contractions. During the provocation, the contractions of the jaw-closing muscles were performed in six sets of exercises, each lasting 5 minutes, with 1 minute of rest in between. Each set of exercises consisted of about 60 open-close movements, each movement lasting about 5 seconds, with the eccentric contraction lasting approximately 3 seconds, the concentric contraction 1 second, and the "occlusal" phase (the transition from closing to opening), lasting 1 second. Individually made mouthguards were used by the participants in order to protect the teeth during the provocation.

NGF Injection

Based on previous studies,[19,20,24] a single dose of 5 µg of NGF was given in 0.2-mL saline as a bolus injection into the masseter muscles of the participants on both sides. The injection was done according to previously published techniques.[19,42]

Assessment of Mechanical Sensitivity and RS

Mechanical sensitivity of the masseter and temporalis muscles was assessed using three different forces (0.5 kg, 1.0 kg, and 2.0 kg) with the aid of palpometers (Palpeter, Sunstar Suisse). Each muscle was palpated at nine different sites (3 × 3 square) in a randomized sequence. The duration of a single-palpation stimulus was 2 seconds and was followed by a 10-second interval to avoid any sensitization of the assessed muscles and to allow the participants to rate the perceived intensity on a 0–50–100 numeric rating scale (NRS; where 0 was no sensation, 1–49 was a sensation that was not painful, 50 was the pain threshold, and 100 was the most painful sensation imaginable) and to report RS, if they experienced any. If RS was present, participants were asked to rate the RS intensity on the same 0–50–100 NRS and to draw its location on an anatomical map of the head. Finally, assessment of mechanical sensitivity in the thenar region as a control site was done with a 2.0-kg palpometer and was repeated three times.

Questionnaires

All participants were asked to complete the OHIP, which evaluates the social impact of oral disorders and masticatory ability; the JFLS, which evaluates jaw functional limitation; and the OBC, which assesses the frequency of jaw overuse behaviors. The OHIP-49 questionnaire includes 7 domains representing functional limitation (9 items), physical pain (9 items), psychologic discomfort (5 items), physical disability (9 items), psychologic disability (6 items), social disability (5 items), and handicap (6 items). A 0–5 scale (0 = never, 1 = hardly ever, 2 = occasionally, 3 = fairly often, 4 = very often, and 5 = all the time) is used for each item, so total scores can range from 0 to 245.[37,43,44] The JFLS has three domains concerning mastication (6 items), vertical jaw mobility (4 items), and emotional and verbal expression (10 items), and uses a scale from 0 (no limitation) to 10 (severe limitation) for each. Total scores can vary from 0 to 200.[38] The OBC comprises 21 questions with a scale of 0–4 (0 = none of the time, 1 = a little of the time, 2 = some of the time, 3 = most of the time, and 4 = all of the time) used for each, and total scores can vary from 0 to 84.[39] Additionally, a 0 (no pain) to 10 (worst imaginable pain) NRS diary was filled out by participants to register the average scores of muscle pain, unpleasantness, tiredness, tension, soreness, and stiffness[40,41] in the morning and afternoon.

Assessment of Oral Motor Function

Maximum Opening

Participants were instructed to open their mouth as wide as possible; first without experiencing any pain, and then even if they experienced pain. Maximum opening was defined as the sum of the distance between the incisal edges of the maxillary and mandibular central incisors on the right side and the overbite when in maximum intercuspation.

Pain on maximum bite and MBF. The MBF was recorded with the use of a bite force transducer.[45] The participants were asked to bite as hard as possible on the bite force meter placed between the first molars of the dominant side. Verbal encouragement was given during the task so that the participants kept biting as hard as possible for approximately 3 seconds. Participants were asked to rate any pain they experienced during the task on a 0–10 NRS. The MBF recording was repeated three times, with 1 minute between recordings to prevent muscle fatigue and to allow recovery. The mean of the three measurements was used for the analysis.

Pain on chewing and chewing efficiency. Three pieces of color-changing chewing gum (70 × 20 × 1 mm; Masticatory Performance Evaluating Gum XYLITOL, Lotte) were given to the participants, and 20 seconds of chewing was conducted on the preferred chewing side. The chewing gum was designed to change from yellow-green to red when chewed. The participants were asked to report any pain they experienced in the masseter and temporalis muscles on a 0–10 NRS during the task. To evaluate the chewing reproducibility, the participants were asked to conduct each chewing test twice, and the average NRS during each chewing task was taken.

After completion of each chewing task, the gum was placed in a transparent plastic bag and flattened to a 1-mm–thick wafer. A digital color image of each side of the chewed gum was photographed using a digital camera under standard lighting with identical luminous intensity, as previously described.[46,47] The chewing efficiency was evaluated by comparing the ratio of red pixels to the total pixels using ImageJ (National Institutes of Health).

Electrognathographic signals and temporal summation. The electrognathographic (EGG) signals were tracked in the anterior-posterior (x), lateral (y), and vertical (z) axes with a craniomandibular evaluation system (Sirognathograph, Siemens). The jaw tracker was calibrated by standard blocks made of plexiglass following previous studies.[48,49] A magnet was placed on the labial side of the mandibular central incisors of each participant, without occlusal interference with the maxillary teeth, in the intercuspal position (ICP). The participants were instructed to sit straight and stare at a fixed spot in the front at eye level to maintain the head posture. Then the jaw-tracker frame and sensor array were placed on

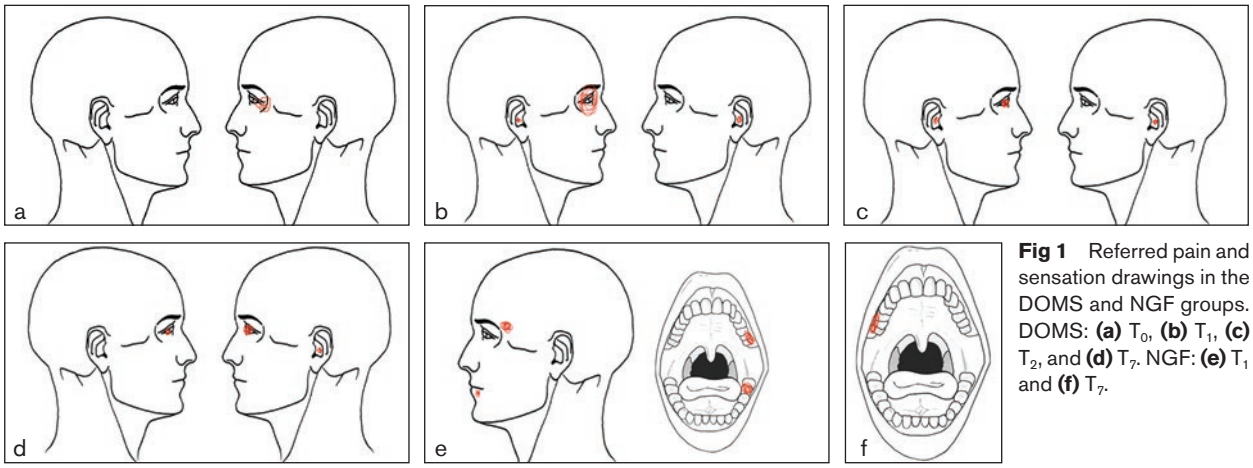

Fig 1 Referred pain and sensation drawings in the DOMS and NGF groups. DOMS: **(a)** T_0, **(b)** T_1, **(c)** T_2, and **(d)** T_7. NGF: **(e)** T_1 and **(f)** T_7.

the head and adjusted to keep the magnet in the middle of the sensor array and the long axis of the magnet and the axes of the sensor array paralleled.

With the equipped jaw tracker connected to the computer and the outputs adjusted to zero (0, 0, 0), the participants, in ICP position, were instructed to open their mouth as wide as possible (even with pain) and then close to ICP repeatedly in a computer-generated rhythm of 70 mm/second. Four continuous jaw movements × 5 sequential repetitions (with a 3-second interval between each repetition) of open-close movements were executed.[32] The participants were instructed to rate the intensity of pain at the end of each repetition on a 0–10 NRS.

Statistical Methods

Data were assessed for normality using quantile-quantile (Q-Q) plots. For the analysis of pain on chewing, pain on maximum bite, and pain diary score, the data were log-transformed to approximate normality and to stabilize the variance.

Two-way repeated-measures analysis of variance (ANOVA) was used to test for the OBC score, mechanical sensitivity at the thenar region, maximum opening (with and without pain), pain on maximum bite, MBF, chewing efficiency, and pain on chewing. The independent factors were: *(1)* group (3 levels: DOMS, NGF, and control) and *(2)* time (4 levels: T_0, T_1, T_2, and T_7).

Three-way repeated-measures ANOVA was performed for analysis of OHIP and JFLS scores, repeated-opening measurement, and activity-related temporal summation of pain. The independent factors were: *(1)* group (3 levels: DOMS, NGF, and control); *(2)* time (4 levels: T_0, T_1, T_2, and T_7); and *(3)* subscale (OHIP [7 levels: functional limitation, physical pain, psychologic discomfort, physical disability, psychologic disability, social disability, and handicap], JFLS [3 levels: mastication, mobility, and expression], repeated-opening measurement [5 levels: repetition 1–5], or activity-related temporal summation of pain).

Four-way repeated measures ANOVA was used to test differences in the pain diary scores. The independent factors were: *(1)* group (3 levels: DOMS, NGF, and control); *(2)* subscale (6 levels: pain, unpleasantness, tiredness, tension, soreness, and stiffness); *(3)* session (2 levels: morning [am] and afternoon [pm]); and *(4)* time (8 levels: T_0, T_1, T_2, T_3, T_4, T_5, T_6, and T_7).

Five-way repeated measures ANOVA was used to test for differences in mechanical sensitivity of the masticatory muscles with the independent factors: *(1)* group (3 levels: DOMS, NGF, and control); *(2)* muscle (2 levels: masseter and temporalis); *(3)* force (3 levels: 0.5 kg, 1 kg, and 2 kg); *(4)* side (2 levels: left and right); and *(5)* time (4 levels: T_0, T_1, T_2, and T_7).

Tukey HSD was used for post hoc testing and correction for multiple comparisons when main effects and/or interactions were significant. All statistical calculations were performed using STATISTICA (version 10.0; StatSoft). The data are presented as mean ± standard error of the mean (SEM), and levels of $P < .05$ were considered statistically significant.

Results

All participants completed all procedures in the experiment.

After 24 hours, all healthy participants in the NGF group were diagnosed with myalgia according to the DC/TMD, whereas 70% of the DOMS group developed myalgia according to the DC/TMD. In the DOMS group, one female participant reported RS in the orofacial region at T_1, T_2 and T_7, and one male participant reported RS at T_0, T_1, T_2, and T_7 (Figs 1a to 1d). In the NGF group, one male participant experienced RS at T_1, and another at T_1 and T_7 (Figs 1e and 1f).

Table 1 shows an overview of gender distribution and age of participants in each group. Table 2 shows the baseline data of the participants for each group and sex.

Table 1 Gender and Age Distribution of Participants

	DOMS		NGF		Control	
	Male	Female	Male	Female	Male	Female
No.	12	8	12	8	6	4
Mean age (range), y	30 (21–62)		27 (21–69)		29 (19–56)	

DOMS = delayed-onset muscle soreness; NGF = nerve growth factor.

Table 2 Baseline Data of the Participants in Each Group

		Mechanical sensitivity (0–50–100 NRS)		Questionnaires (total score)			Maximum opening with pain, mm	Maximum biting force, N	Chewing efficiency, %
		Masseter	Temporalis	OHIP	JFLS	OBC			
DOMS	M	25 ± 4	20 ± 4	18 ± 4	1 ± 1	16 ± 3	57 ± 1.6	527 ± 83.5	97.7 ± 0.4
	F	32 ± 6	22 ± 5	25 ± 8	6 ± 4	22 ± 4	48.9 ± 2.2	338.4 ± 50.8	98.1 ± 0.5
NGF	M	31 ± 3	20 ± 3	36 ± 11	4 ± 1	20 ± 2	57.8 ± 1.8	578.5 ± 53	98.4 ± 0.4
	F	33 ± 6	24 ± 6	35 ± 6	2 ± 1	23 ± 4	44.5 ± 2	433.4 ± 46.4	97.7 ± 0.5
Control	M	28 ± 5	22 ± 5	22 ± 6	2 ± 1	23 ± 5	58.7 ± 3.5	622 ± 85.9	97.8 ± 0.5
	F	30 ± 9	16 ± 5	13 ± 6	0 ± 0	14 ± 4	52.4 ± 3.3	401.4 ± 54.9	98.6 ± 0.3

Data are reported as mean ± SE. DOMS = delayed-onset muscle soreness; NGF = nerve growth factor; OHIP = Oral Health Impact Profile; JFLS = Jaw Functional Limitation Scale; OBC = Oral Behaviors Checklist.

Self-Reported Muscle Symptoms

There was a statistically significant effect of time (ANOVA, F = 11.52, df = 7, $P < .001$) and a significant interaction between subscale and group (ANOVA, F = 2.48, df = 10, $P = .008$), as well as among session, time, and group (ANOVA, F = 4.35, df = 14, $P < .001$). Post hoc tests showed that there were increased ratings between T_0pm and T_3pm compared to T_0am, with the peak occurring at T_1pm in the DOMS group and increased ratings between T_0pm and T_7pm compared to T_0am in the NGF group, with the peak at T_3pm ($P < .001$). In the NGF group, the scores increased between T_2am and T_5am when compared to the control group, and increased between T_2pm and T_4pm compared to the DOMS group (Tukey post hoc, $P \leq .035$) (Fig 2).

Mechanical Sensitivity

Masseter and temporalis. There were statistically significant main effects for muscle (ANOVA, F = 83.3, df = 1, $P < .001$), force (ANOVA, F = 261.4, df = 2, $P < .001$), and time (ANOVA, F = 14.4, df = 3, $P < .001$), as well as significant interactions among muscle, time, and group (ANOVA, F = 9.9, df = 6, $P < .001$) and among force, time, and group (ANOVA, F = 5.2, df = 12, $P < .001$). Post hoc tests showed that masseter muscle mechanical sensitivity was increased at T_1 and T_2 in the DOMS group, and for a longer period (T_1, T_2, and T_7) in the NGF group, when compared to baseline ($P < .001$) (Fig 3). Compared to T_0, increased mechanical sensitivity was found at T_1, T_2, and T_7 with 0.5-kg and 1-kg palpation and at T_2 with 2-kg palpation in the NGF group, and at T_1 and T_2 with 2-kg palpation in the DOMS group (Tukey post hoc, $P < .001$).

Thenar. There was no significant main effect nor any significant interaction in the thenar region (ANOVA, $P > .05$).

Questionnaires

Regarding the OHIP, there was a statistically significant main effect of group (ANOVA, F = 3.35, df = 2, $P = .044$), time (ANOVA, F = 4.18, df = 3, $P = .007$), and subscale (ANOVA, F = 30.39, df = 6, $P < .001$), and a significant interaction among time, subscale, and group (ANOVA, F = 1.68, df = 36, $P = .008$). Post hoc tests showed that OHIP scores were increased at T_1 and T_2 in the NGF group when compared to T_0 ($P \leq .042$) and at T_2 when compared to the control group ($P = .014$) in the subscale of physical pain (Fig 4). As such, the OHIP scores showed that an increase in physical pain in the NGF group mainly took place 2 days after the injection.

For the JFLS, there was a statistically significant main effect of time (ANOVA, F = 13.38, df = 3, $P < .001$) and a significant interaction among subscale, time, and group (ANOVA, F = 2.88, df = 12, $P < .001$). Post hoc tests showed that JFLS scores for mastication increased at T_1, T_2, and T_7 in the NGF group compared to T_0 ($P < .001$) and at the same time points when compared to the DOMS ($P \leq .024$) and control groups ($P < .001$). At T_1, the mastication scores were also increased for the DOMS

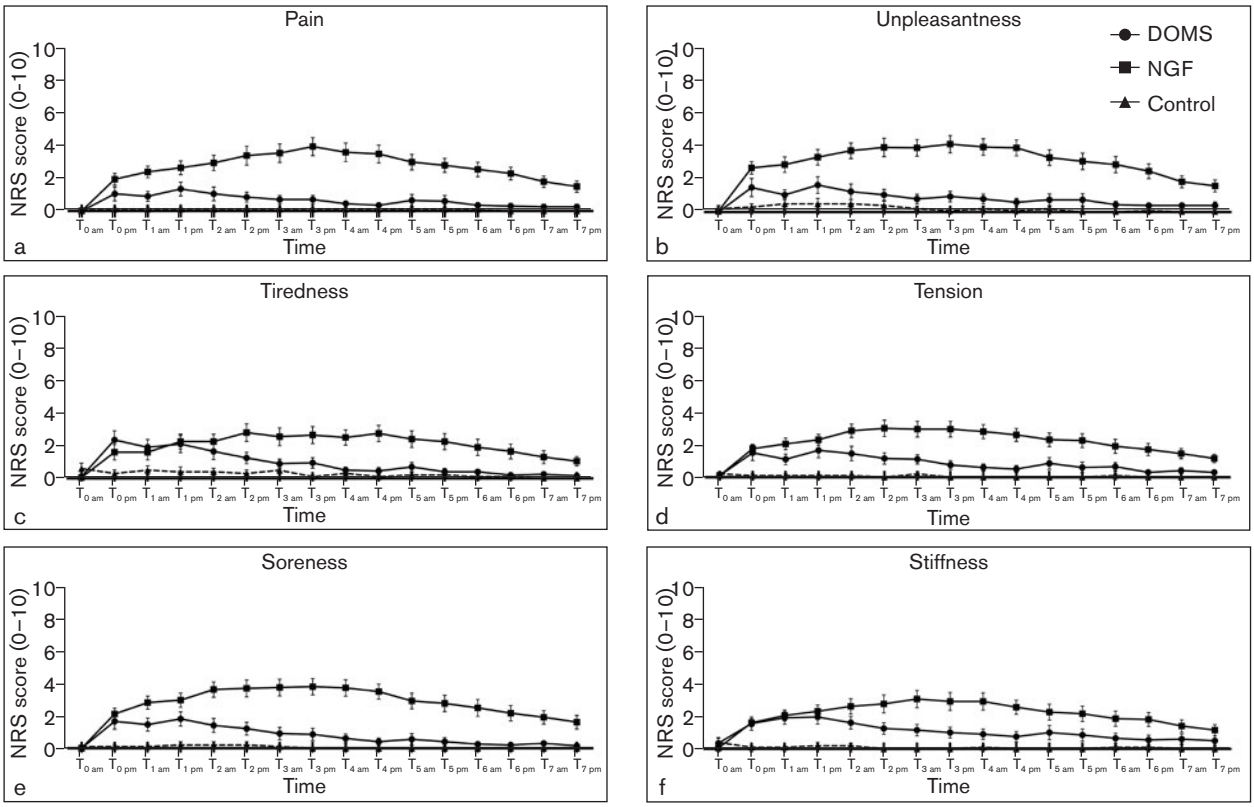

Fig 2 Self-reported mean ± SE values of **(a)** pain, **(b)** unpleasantness, **(c)** tiredness, **(d)** tension, **(e)** soreness, and **(f)** stiffness in the DOMS, NGF, and control groups. Compared to T_0am, the mean scores of pain increased from T_0pm to T_3pm, with the peak at T_1pm, in the DOMS group, and increased from T_0pm to T_7pm, with the peak at T_3pm, in the NGF group ($P < .001$). In the NGF group, the score between T_2am and T_5am also increased when compared to the control group and increased between T_2pm and T_4pm when compared to the DOMS group (Tukey post hoc, $P \leq .035$).

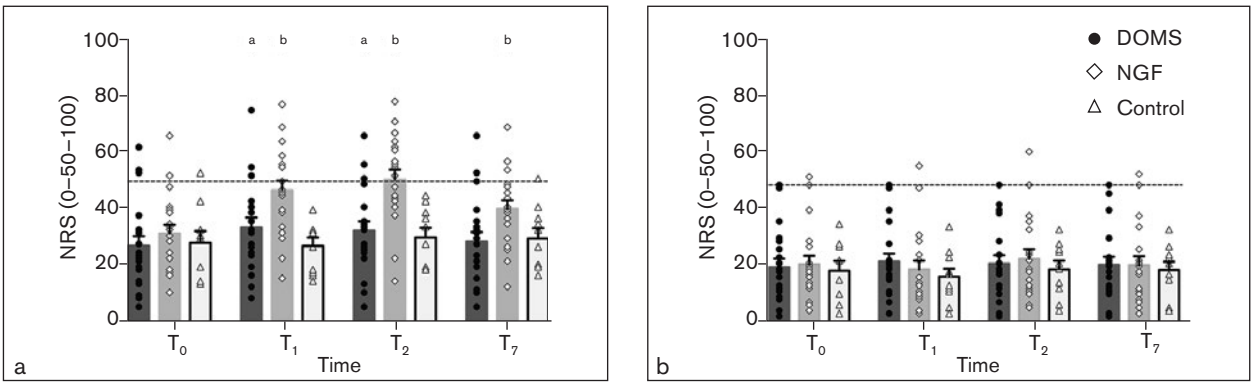

Fig 3 Mean ± SE values of muscle sensitivity of the **(a)** masseter and **(b)** temporalis muscles in the DOMS, NGF, and control groups. [a]Significant increase compared to baseline in DOMS group ($P < .001$). [b]Significant increase compared to baseline in NGF group ($P < .001$).

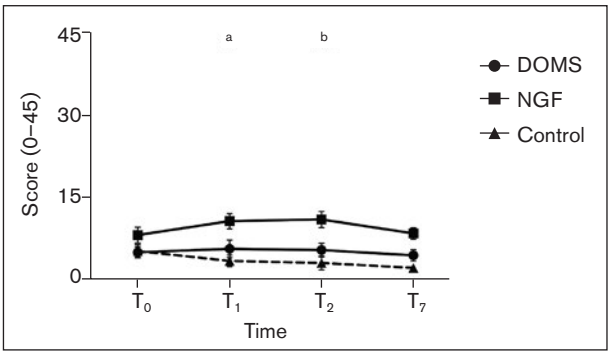

Fig 4 Mean ± SE score for Oral Health Impact Profile (OHIP) subscale of physical pain. [a]Significant increase of score in NGF group compared to baseline ($P = .042$). [b]Significant increase of score in NGF group compared to baseline ($P = .005$) and at the same time point when compared to control group ($P = .014$).

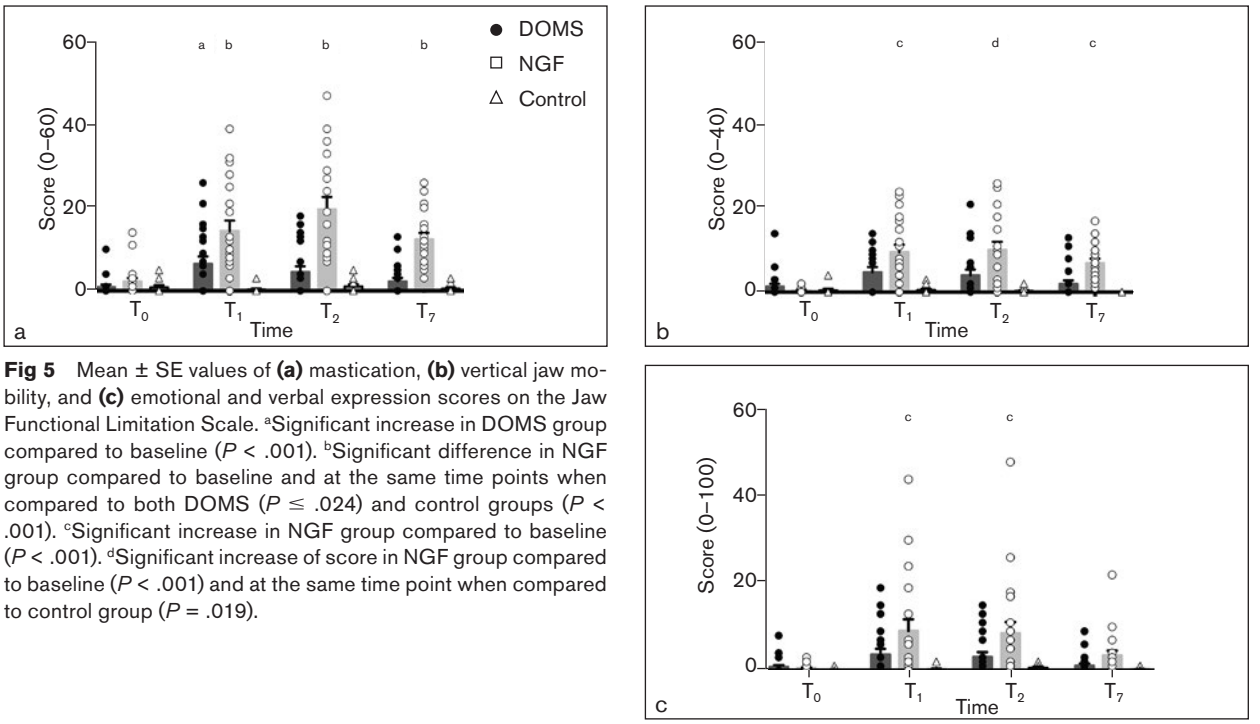

Fig 5 Mean ± SE values of **(a)** mastication, **(b)** vertical jaw mobility, and **(c)** emotional and verbal expression scores on the Jaw Functional Limitation Scale. [a]Significant increase in DOMS group compared to baseline ($P < .001$). [b]Significant difference in NGF group compared to baseline and at the same time points when compared to both DOMS ($P \leq .024$) and control groups ($P < .001$). [c]Significant increase in NGF group compared to baseline ($P < .001$). [d]Significant increase of score in NGF group compared to baseline ($P < .001$) and at the same time point when compared to control group ($P = .019$).

group when compared to T_0 ($P < .001$) (Fig 5a). Post hoc tests also showed that, in the NGF group, JFLS scores for vertical jaw mobility were increased at T_2 when compared to the control group ($P = .019$) and increased at T_1, T_2, and T_7 when compared to T_0 ($P < .001$) (Fig 5b), and that JFLS scores for the verbal and emotional scales increased at T_1 and T_2 compared to T_0 ($P < .001$) (Fig 5c).

There were no significant main effects nor interactions in OBC scores (ANOVA, $P > .05$).

Maximum Opening

There was a statistically significant main effect of time (ANOVA, F = 14.14, df = 3, $P < .001$) and a significant interaction between time and group (ANOVA, F = 3.77, df = 6, $P = .002$) for maximum opening without pain. Post hoc tests showed that maximum opening without pain was decreased in the NGF group at T_1 and T_2 ($P < .001$) compared to T_0 (Fig 6). The values of maximum opening without pain in all three groups are shown in Table 3a.

There was no significant main effect nor any significant interaction for maximum opening with pain (ANOVA, $P > .05$).

Maximum Bite Assessment

Pain on maximum bite. There was a statistically significant main effect of time (ANOVA, F = 7.065, df = 3, $P < .001$) and a significant interaction between time and group (ANOVA, F = 4.266, df = 6, $P < .001$). Post hoc tests showed that, in the NGF group, pain on maximum bite was increased at T_1 and T_2 compared to T_0 and also increased at T_2 compared to the control group ($P \leq .011$).

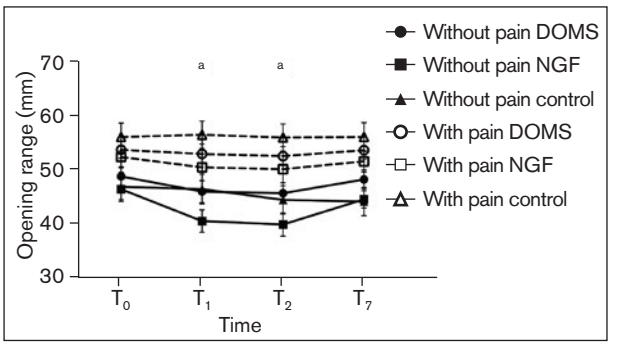

Fig 6 Mean ± SE values of maximum opening range in the DOMS, NGF, and control groups. [a]Significant decrease in opening range in NGF group compared to baseline ($P < .001$).

Maximum bite force. There were no significant main effects nor interactions in MBF (ANOVA, $P > .05$). Table 3b shows the MBF values of all three groups.

Chewing

Pain. There were statistically significant main effects of group (ANOVA, F = 10.02, df = 2, $P < .001$) and time (ANOVA, F = 13.75, df = 3, $P < .001$), and a significant interaction between time and group (ANOVA, F = 4.37, df = 6, $P < .001$). Post hoc tests showed that pain on chewing was increased at T_2 compared to T_0 in the DOMS group ($P = .035$). The increase in pain mainly took place in the NGF group, which

Table 3a Descriptive Statistics of Maximum Opening Without Pain (mm)

		Baseline (T_0)	1 d (T_1)	2 d (T_2)	7 d (T_7)
DOMS	M	53.1 ± 1.5	49.7 ± 2.4	50.4 ± 1.8	52.5 ± 1.7
	F	42.5 ± 2.5	40.6 ± 2.8	38.9 ± 2.5	42.1 ± 2.2
NGF	M	50.6 ± 1.9	45.0 ± 1.9	44.7 ± 2.0	48.8 ± 1.5
	F	40.4 ± 2.5	34.1 ± 3.3	33.0 ± 3.1	38.5 ± 1.7
Control	M	48.2 ± 3.4	49.2 ± 2.7	46.5 ± 1.4	45.7 ± 2.1
	F	45.1 ± 4.6	42.6 ± 5.5	41.6 ± 6.4	42.1 ± 6.2

Data are reported as mean ± SE.

Table 3b Descriptive Statistics of Maximum Bite Force (N)

		Baseline (T_0)	1 d (T_1)	2 d (T_2)	7 d (T_7)
DOMS	M	527.0 ± 83.5	549.3 ± 85.2	579.1 ± 92.5	622.1 ± 97.1
	F	338.4 ± 50.8	298.1 ± 39.1	383.5 ± 40.0	432.2 ± 60.0
NGF	M	578.5 ± 53.0	580.9 ± 56.2	575.3 ± 62.1	566.3 ± 56.8
	F	433.4 ± 46.4	418.6 ± 43.2	430.6 ± 61.4	439.0 ± 61.5
Control	M	622.0 ± 85.9	551.5 ± 81.9	567.9 ± 56.6	575.4 ± 78.2
	F	401.4 ± 54.9	450.7 ± 83.2	456.1 ± 56.9	473.0 ± 55.2

Data are reported as mean ± SE.

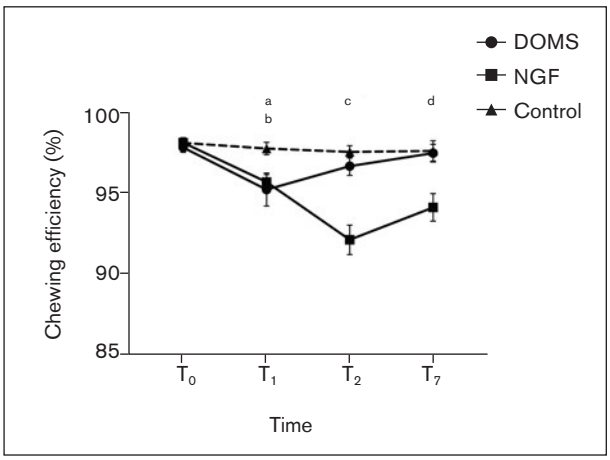

Fig 7 Mean ± SE chewing efficiency in the DOMS, NGF, and control groups. [a]Significant difference compared to T_0 in NGF group ($P \leq .031$). [b]Significant difference compared to T_0 in DOMS group ($P = .013$). [c]Significant difference in NGF group compared to baseline and at the same time point when compared to DOMS and control groups ($P < .001$). [d]Significant difference in NGF group compared to baseline ($P < .001$) and at the same time point when compared to DOMS group ($P = .007$).

showed increased values at T_1 and T_2 compared to T_0 and compared to the same time points in the DOMS and control groups (Tukey post hoc, $P \leq .004$).

Chewing Efficiency

There were statistically significant main effects of group (ANOVA, F = 7.62, df = 2, $P = .001$) and time (ANOVA, F = 10.43, df = 3, $P < .001$) and a significant interaction between time and group (ANOVA, F = 7.74, df = 6, $P < .001$). Post hoc tests showed there was a decrease in chewing efficiency at T_1 compared to T_0 in the DOMS group ($P = .013$). In the NGF group, there was a decrease at T_1, T_2, and T_7 compared to T_0, and a decrease at T_2 compared to the DOMS and control groups, and a decrease at T_7 compared to the DOMS group (Tukey post hoc, $P \leq .031$) (Fig 7). The NGF group had a more profound decrease in chewing ability, which lasted longer compared to the DOMS group.

Repeated-Opening Measurement and Repetition-Induced, Activity-Related Summation

Activity-related temporal summation of pain. There was a statistically significant main effect of repetition (ANOVA, F = 8.72, df = 4, $P < .001$) and a significant interaction between time and group (ANOVA, F = 6.43, df = 6, $P < .001$). No significant differences were seen for the interaction between group and repetition, which would have indicated differences in activity-related temporal summation of pain.

Repeated-opening measurement. There was a statistically significant main effect of time (ANOVA, F = 13.72, df = 3, $P < .001$) and a significant interaction between time and group (ANOVA, F = 3.3, df = 6, $P = .005$). Post hoc tests showed that, compared to T_0, repeated opening was decreased at T_1 and T_2

($P < .001$) in the DOMS group and decreased at T_1, T_2, and T_7 in the NGF group ($P < .001$).

Discussion

The main findings in this study were that both experimental pain models caused increases in mechanical sensitivity of the masseter muscle, pain on chewing, and decreases in chewing efficiency when compared to the control group, with the effects of the NGF model lasting longer than the DOMS model. Furthermore, the NGF model caused disability in parameters not significantly affected by the DOMS model, such as subjective jaw function disability, maximum opening without pain, and pain on maximum bite. Finally, none of the models caused an increase in repetition-induced, activity-related temporal summation of pain.

The Pain-Adapted DOMS and NGF Models

The NGF model affected more of the assessed parameters than the DOMS model. In addition, in the parameters that were affected by both models (such as mechanical sensitivity, pain on chewing, and chewing efficiency), the NGF model had a longer duration.

Regarding the NGF model, this study confirmed the results from previous studies showing that NGF injected into the masseter muscle increases mechanical sensitivity and disability during jaw function and decreases jaw-opening ability.[20,50] On the other hand, the results from the present study did not confirm some of the results from previous studies in which the DOMS model was used. The main differences were that, in the present study, DOMS did not lead to decreases in pain-free jaw-opening ability, nor did it cause increases in fatigue and pain, as have been previously reported.[16,18,26] However, a more recent study also did not find increases in pain following application of the DOMS model.[18] The reasons for the differences between the studies could be due to the design of the study, as, for example, this study had three different groups (NGF, DOMS, and control), while the other studies did not have a control group. On the other hand, it could be that the DOMS model technique is operator-sensitive, and, as such, results differ depending on the operator.

The NGF model caused pain on chewing for 2 days after the intramuscular injection, which is in accordance with a previous study.[20] Furthermore, it also caused impairment in mastication ability that lasted 7 days. In contrast, the DOMS model showed either decreased chewing efficiency or increased pain on mastication within 2 days after the provocation. Previous research has shown that in myalgic TMD patients exposed to a chewing task, two subgroups emerge: one in which pain increases and another in which pain decreases. This might be explained by different pathologies or pre-exercise pain levels.[51] In the present study, it is possible that the differences in both pain and motor function outcomes could be due to different pre-exercise pain or sensitization levels.

In summary, the results showed that the NGF model seems to more closely resemble the pain experience of a TMD patient than the DOMS model, since it affects the same parameters that would be affected in a TMD patient, such as pain on palpation, decrease in pain-free jaw opening, and jaw functional ability.[52] Moreover, its effects last longer, which makes it more suitable for assessing long-term effects of myalgia than the DOMS model. Despite this, the DOMS model is a very viable economical substitute for situations where one is mainly interested in studying mechanical sensitivity of the masticatory muscles and pain on chewing compared to the more expensive NGF option. In addition, an animal study has shown that the NGF-induced myalgia with enhanced response of muscle nociceptors may be mediated through increasing expression of the peripheral receptor,[53] while the DOMS model most likely causes masticatory myalgia by inducing inflammation due to microtrauma and the accumulation of histamines, prostaglandins, and potassium.[54] Therefore, the two models can be used to study the pathophysiology of masticatory myalgia in two different forms.

Activity-Related Temporal Summation of Pain

Temporal summation, presumed to be the psychophysical manifestation of "wind-up," is a phenomenon of increased pain perception in response to a series of repetitive noxious stimuli.[55,56] The underlying mechanism of "wind-up" refers to the increased excitability of dorsal horn or trigeminal nucleus caudalis neurons elicited by the repetitive stimulation of C fibers with sufficient frequency and intensity.[57–60] It has been suggested that temporal summation of pain could be increased through the influence of psychologic aspects, such as fear of pain, by altering the pain-inhibitory system or via central modulation of affective processes involved in pain.[30,61,62]

In the present study, activity-related temporal summation of pain was not enhanced in either the DOMS or NGF models. This is contrary to a previous study showing that activity-related temporal summation of pain was increased in temporomandibular joint (TMJ) arthralgia patients when compared to healthy controls.[32] This seems to indicate that the mechanisms involved in pain induction in both of the pain models, at least up to 7 days, are mainly peripheral, whereas in chronic TMD patients, there is central nervous system (CNS) involvement as well as a peripheral component.

A possible explanation could be that either the neurophysiologic mechanisms of the pain models differ from those of actual TMD patients or that the duration of pain in these pain models was not sufficient to cause involvement of the CNS. Another explanation could be a difference in the affective component between chronic pain patients and experimental pain models. It has been shown that increased activity-related temporal summation of pain in patients with fibromyalgia is positively correlated with fear of movement rather than with generalized hyperalgesia.[30,31] As such, it is hypothesized that the lack of activity-related temporal summation of pain in the present study could be due to the short-term pain experience when compared to chronic pain patients. Further studies are needed to evaluate the influence that the affective components of pain, such as fear of movement, may have on activity-related temporal summation of pain in experimental pain models of the masticatory muscles. Therefore, it may be proposed that for future studies assessing activity-related temporal summation of pain in experimental masticatory muscle pain models, a longer duration of pain than what was shown in this study is required. This could be achieved by, for example, repeated injections of NGF.[50,63]

One of the limitations of this study is that for pain scores during maximum bite, participants also reported simultaneous pain from the teeth or gums, which could lead to difficulties in rating pain from the muscles rather than from the teeth or gums. A further limitation is that RS was assessed with 2 seconds of palpation rather than the 5 seconds recommended by the DC/TMD,[36] which could explain the low RS frequency when compared to previous studies.[50,64,65] This was done because it has been shown that palpation for 5 seconds causes significantly increased mechanical sensitivity compared to palpation for 2 seconds. Genders were also not separately analyzed due to the small sample size; as such, the involvement of a larger number of participants is necessary in future studies when sex differences are taken into consideration.

Conclusions

Both NGF and DOMS models produced similar pain-related outcomes, with the NGF model having a longer effect. However, the NGF model showed a more pronounced effect on motor function. Since both models cause pain through different mechanisms and affect the masticatory muscles in different forms, each could be used to study the pathophysiology of different types of masticatory myalgia. Finally, neither of the two models was able to provoke activity-related temporal summation of pain, which indicates that both models elicit pain mainly through peripheral mechanisms rather than central mechanisms.

Key Findings/Highlights

- Both the NGF and DOMS models elicit pain mainly through peripheral mechanisms rather than central mechanisms.
- The two models can be used to study the pathophysiology of different types of masticatory myalgia.

Acknowledgments

This work was funded by the Section of Orofacial Pain and Jaw Function, Department of Dentistry, Aarhus University, the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD, 2018-87) and the Chinese Government Scholarship Fund from China Scholarship Council (201708320348). The authors thank all staff of the Section of Orofacial Pain and Jaw Function, Department of Dentistry and Oral Health, Aarhus University for their selfless help. They would also like to thank Bente Haugsted for help with preparing the NGF injections, Akiko Shimada for providing the chewing gum, and Karina H. Bendixen for her contribution to the study. The authors declare no conflicts of interest.

P.S., M.K., F.G.E., Y.Z.: contributed to study design and acquisition of data; F.G.E., Y.Z.: drafted the article. All authors revised the article for important intellectual concent.

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