Thresholds, Firing Rates, and Order of Recruitment of Anterior Temporalis Muscle Single-Motor Units During Experimental Masseter Muscle Pain

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Aims: To test the hypothesis that, in comparison with control, experimental noxious stimulation of the right masseter muscle would result in significant changes in the firing rates, thresholds, and recruitment orders of single-motor units (SMUs) of the nonpainful, synergistic right anterior temporalis muscle during goal-directed isometric biting task performance. **Methods:** Twenty healthy volunteers received an infusion of hypertonic saline (HS; 5% sodium chloride) into the right masseter to produce pain intensity of 40 to 60 on a 100-mm visual analog scale (VAS). Isotonic saline (IS) infusion was a control. Standardized biting tasks were performed with an intraoral force transducer, and intramuscular electromyographic activity was recorded from the right anterior temporalis muscle. Tasks (slow and fast ramp biting tasks, two-step biting task) were performed in 3 blocks: baseline, HS infusion, and IS infusion. Across blocks, SMU thresholds and firing rates were statistically compared, and SMU recruitment sequences were qualitatively compared. Statistical significance was set at $P < .05$. **Results:** No significant differences ($P > .05$) were noted between HS and IS infusion blocks in thresholds or firing rates of anterior temporalis SMUs. Individual SMUs showed increases or decreases in thresholds or firing rates or changes in recruitment sequences mostly during HS compared to IS infusion. **Conclusion:** The reorganization of SMU activity that has been suggested to occur in both painful and nonpainful agonist jaw muscles may involve not only recruitments and de-recruitments of SMUs, but may also extend to more subtle increases and/or decreases in firing rates, thresholds, and recruitment sequences of individual SMUs in the nonpainful synergistic muscles. *J Oral Facial Pain Headache 2021;35:93–104. doi: 10.11607/ofph.2719*

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Temporomandibular disorders (TMD) are the most prevalent chronic pain condition within the orofacial area,[1] and much has been written about the role of muscle activity in their etiology.[2–5] However, the exact relationship between pain and motor activity is far from understood, and a number of different proposals have been put forward to explain the relationship.[3,5–7] Earlier theories advocated that increases in the activity of agonist muscles lead to spasm and further pain and dysfunction (the Vicious Cycle Theory [VCT]), or that decreases in activity of the agonist muscles are an adapted response to prevent further damage (the Pain Adaptation Model [PAM]).[2,4] However, more recent evidence from limb, neck, and trunk muscle studies,[7–14] as well as jaw muscle studies,[15–25] does not fit well with these earlier theories proposing generalized changes in muscle activity, but is rather more consistent with theories espousing a reorganization of motor-unit activity with the objective of maintaining motor function despite the noxious stimulation. These ideas have been formulated into newer theories—the New Theory of Adaptation to Pain (NTAP)[7] and the Integrated Pain Adaptation Model (IPAM)[26]—that propose a reorganization of activity with recruitment and de-recruitment of single-motor units (SMUs) within painful and nonpainful muscles. The IPAM further suggests that this reorganization is modulated by the individual-specific psychosocial aspects associated with a painful experience.[8,26–28]

In the spinal motor system, in addition to the recruitments and derecruitments of motor unit activity, significant reductions in firing rates have been reported for those SMUs that remained active during both experimental pain and control conditions.[8] In these studies, the nociceptive input appears to be modifying the excitatory and/or inhibitory inputs to the motoneurons through changes in segmental inputs and/or through changes in descending inputs from higher centers. In the jaw motor system, evidence has been provided for recruitments and de-recruitments of temporalis and masseter SMUs during masseter noxious stimulation.[16,24,25] These recruitments and de-recruitments of motor units reflect changes to the synaptic excitation (ie, drive) of the motoneurons that are sufficiently large to excite the motoneuron (and result in motoneuron recruitment, recordable as an SMU action potential) or to silence the motoneuron (and result in motoneuron de-recruitment, recordable as a silencing of the firing of an SMU action potential).

Nociceptive activity may also result in more subtle changes to the excitatory drive of motoneurons that may be reflected in changes to the thresholds, firing rates, and/or sequences of recruitment of those SMUs that remain active under both pain and control conditions. In previous studies of experimental masseter noxious stimulation in the jaw motor system, reductions in masseter SMU firing rates (increases in inter-spike intervals) were noted in one study,[29] and both significant increases and decreases in SMU firing rates were noted in another study.[24] Previous studies in the spinal motor system have reported changes in recruitment patterns during experimental pain in comparison with control.[9]

The previous, related study[25] provided evidence for recruitments and de-recruitments of SMUs not only in the painful jaw muscle as previously noted,[16,24] but also in the nonpainful synergistic temporalis muscle.[25] While there is evidence from previous studies that noxious masseter muscle stimulation can result in recruitments and de-recruitments of masseter SMUs, as well as more subtle changes in the firing properties of masseter SMUs that remain active during pain in comparison with control,[16,24,29] there are no data sets addressing whether such subtle changes in SMU-firing properties might also occur in the nonpainful synergistic muscles. Therefore, the aim of the present study was to test the hypothesis that, in comparison with control, experimental noxious stimulation of the right masseter muscle results in significant changes in the firing rates, thresholds, and recruitment orders of SMUs of the nonpainful, synergistic right anterior temporalis muscle during a goal-directed isometric biting task performance.

Materials and Methods

Twenty healthy participants voluntarily agreed to the study (15 women, 5 men; age range: 22–40 years; mean [SD]: 29.5 [4.3] years). All volunteer students from the University of Sydney and the general public signed informed consent forms before the study. The methodology and procedures were approved by the Western Sydney Local Health District Human Ethics Committee of Westmead Hospital (Western Sydney Local Health District: HREC2003/8/3.2[1645]) and the Human Ethics Committee of the University of Sydney (2479). The sample size was based on previous studies in both the spinal and trigeminal literature[8,16,24,29,30] that had demonstrated changes in SMU recruitment patterns with comparable sample sizes of participants, as well as the number of SMUs discriminated.

A calibrated examiner (T.W.) used the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD)[31] to exclude participants with a diagnosis of TMD. The RDC/TMD was used in this study instead of the Diagnostic Criteria for TMD (DC/TMD)[1] because the study commenced in the year that the DC/TMD was first published, and the RDC/TMD was used only to rule out TMD. Other exclusion criteria were: pain during muscle palpation; history of chronic pain; ongoing chronic or acute pain conditions; medications for chronic diseases; neuromuscular dysfunction or systemic musculoskeletal conditions; pregnancy; high blood pressure; a large overjet or overbite that could have impeded the force transducer placement; < 24 teeth; use of denture(s); and active orthodontic treatment. The study was carried out over two sessions with the participants at the Jaw Function and Orofacial Pain Research Unit (Westmead Centre for Oral Health, Sydney). Many of the procedures have been previously described in detail.[19,25] Two previous papers were published with data collected in the same experimental sessions as in the present study.[19,25] The first paper[19] reported bilateral surface EMG activity of masseter and anterior temporalis muscle activity during painful and nonpainful vertical biting tasks. The other paper[25] presented data regarding the recruitment and de-recruitment of SMUs of the right masseter and right anterior temporalis muscles during painful and nonpainful vertical biting tasks. As most of the methods are covered in detail in the previous papers, a summary of the methods will be provided.

In the first session (1 to 1.5 hours), impressions of both dental arches were taken to allow the construction of rigid intraoral splints (Erkoplast-0, 1.5-mm thickness, 120-mm diameter, Erkodent) that housed the bite force transducer (LMA-A, Kyowa)[27–29] to measure vertical jaw closing force. The second (final) session with the participants was the experimental recording session, which lasted approximately 3 to 4.5 hours.

Intramuscular Electrode Placement

Bipolar Teflon-coated fine-wire electrodes with 0.5 mm of exposed wire at the tip (A-M Systems) were placed in the temporalis muscle approximately 1 cm behind the posterior border of the frontal process of the zygoma and also within the central part (horizontally and vertically) of the masseter muscle, with the needle angled downwards at ~30 degrees in relation to the ramus. For the temporalis, the needle was angled downwards at ~45 degrees. To accomplish an accurate electrode placement, repetitive clenches aided the identification of the most active region within each muscle. A ground electrode was also secured to the left wrist. The intramuscular EMG activity was amplified (×5,000–10,000), filtered (bandwidth 100–10 kHz), and digitized (sampling rate: 20,000 samples/second; Micro1401 and Model 1902 Quad System, Cambridge Electronic Design) for subsequent offline discrimination of SMUs.

Induction and Assessment of Pain

A 22-gauge needle-integrated IV catheter was placed into the center of the right masseter muscle and then connected through a polyethylene extension set to an infusion pump (IVAC Model P2000, Alaris) that held a 10-mL syringe containing either hypertonic saline (HS; 5% sodium chloride [NaCl]) or isotonic saline (IS; 0.9% NaCl). The HS infusion rate was controlled between 4 and 6 mL/hour to maintain a moderate pain intensity of between 40 and 60 mm on a 100-mm visual analog scale (VAS), where 0 mm represented "no pain" and 100 mm represented "the worst pain possible." An ongoing moderate level of pain was attained by monitoring the VAS score after each trial and adjusting the infusion rate, if necessary, by 1 to 4 mL/hour. Isotonic saline was infused using the same catheter after attaching a new extension set and was infused at a rate of 4 to 6 mL/hour, or, if infused second, at rates that matched the rates used for the previous HS infusion in that participant. The sequence at which the saline solution was first administered was alternated from one participant to the next, and although participants were not informed which solution would be infused first, they quickly became aware during the infusion. Blinding of the experimenters was not possible in this experimental design because of the wide variability in pain intensity scores between participants, particularly from the hypertonic saline infusion when it was necessary to adjust the infusion rate so that each participants' pain intensity was in the range of 40 to 60 mm.

Biting Tasks

With the force transducer in place, all participants performed three sets of isometric jaw biting tasks, and each performance of a task was named as a trial. All trials included a 2- to 3-second rest period before the participant commenced biting.

- The slow ramp biting task required an increase in jaw biting force to a maximum of 93 N at a low force rate (5 N/second), followed by a prompt reduction of force generation to a new rest period. The task lasted about ~40–45 seconds.
- The fast ramp biting task consisted of an increase in jaw biting force to a maximum of 119 N at a higher force rate (17 N/second), also followed by a prompt reduction of force generation until a new rest period. It took approximately 20 seconds to complete.
- The two-step biting task involved a rapid increase in bite force to a first level (step 1), which was maintained for 2 to 3 seconds. Then, force was rapidly increased again to a higher biting force and maintained for 2 to 3 seconds (step 2), and then there was a rapid decrease until the next resting period.

The chosen force level for step 1 in each participant was the force intensity at which there was evidence of at least one single-motor unit in the EMG recording. The higher force level for step 2 was determined when there was additional recruitment of SMUs and/or an increased firing rate of existing SMUs. The same force values for step 1 and step 2 were used throughout all blocks for the two-step biting task in that participant. Step tasks took about 10 to 12 seconds, with 0.5 to 1 second for every increase in the force level from rest to step 1, and from step 1 to step 2.

The three isometric biting tasks were repeated three times each; therefore, there were nine trials for each of the three blocks:

- Block 1: baseline, before any infusion
- Block 2: trials performed with HS or IS infusion (the order was alternated between participants)
- Block 3: trials performed with IS or HS infusion (the order was alternated between participants)

While a block 4 was performed in which another complete set of trials was performed without any infusion and 10 minutes after removal of the catheter, there was no analysis carried out on block 4 trials for thresholds, firing rates, or orders of recruitment of SMUs recorded. Only the SMU data relating to SMU occurrences were analyzed from block 4, and these data have already been published.[25]

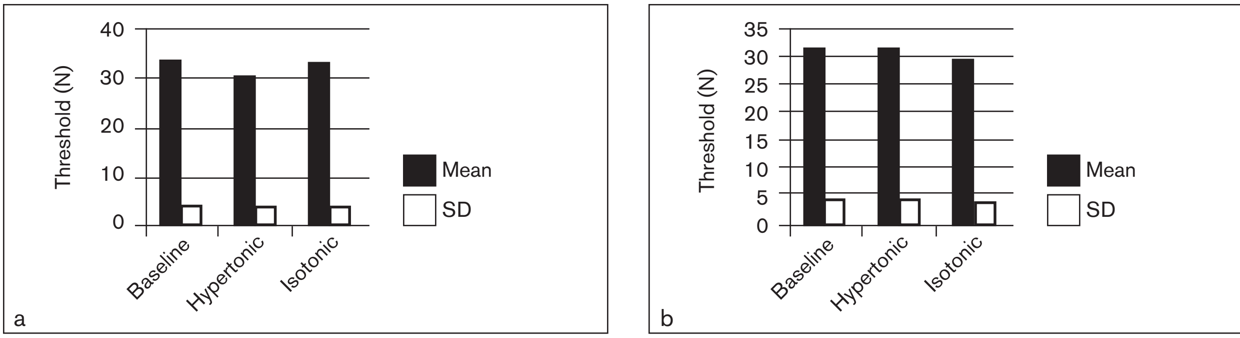

Fig 1 Mean (black histograms) and SD (white histograms) for threshold of onset of SMU activity in the **(a)** slow ramp and **(b)** fast ramp biting tasks under baseline, hypertonic saline infusion, and isotonic saline infusion.

Data Analysis

The data acquired during both saline infusion blocks were grouped as data sets for the HS infusion block and for the IS infusion block. SMU activity was discriminated in the right anterior temporalis muscle for each trial of the three biting tasks (slow ramp biting task, fast ramp biting task, and two-step biting task). For both ramp biting tasks, SMU discrimination was carried out prior to the onset of force increase in each trial until SMU detection was no longer possible more during the ramp task. For the two-step biting task trials, the most stable 2-second period (lowest SD) defined for each step was used to determine the period over which SMUs were discriminated. Discrimination of SMUs was possible by employing template-matching software (Spike2, Cambridge Electronic Design) that allowed visual confirmation of SMUs with similar amplitude and shape. However, due to technical issues (eg, excessive background noise, signal was lost), some SMUs were unable to be discriminated in some trials.

Calculation of the threshold for onset of firing for each SMU in relation to force (Newtons) was performed for each slow and fast ramp biting task trial. The threshold value was the level of force (in N) when an SMU became active continuously (ie, at least 5 action potentials per second) for at least 1 second. All data sets were confirmed to follow a normal distribution (Shapiro-Wilk test, $P > .05$). Repeated-measures analysis of variance (ANOVA) with Bonferroni correction was carried out to investigate the effect of pain on the SMU threshold values among the three blocks.

An individual qualitative SMU analysis was also performed in order to determine the direction of the change of individual threshold values. If the difference in mean threshold between HS and IS was > 0.5 N, then the threshold was determined to have increased (+) during HS, and if the difference in mean threshold between HS and IS was ≤ 0.5 N, then the threshold was determined to have decreased (–) during HS. If the absolute value of the change in threshold between HS and IS was 0.5 N, then the threshold was determined not to have changed (o). The purpose of this qualitative analysis was to provide preliminary data as to whether future studies could address a more detailed individual SMU threshold analysis.

The data from the slow and fast ramp biting tasks were further analyzed to determine whether the sequence of recruitment of the SMUs was altered when comparing baseline, HS, and IS. For this purpose, only SMUs that were present in the baseline, HS, and IS were considered. The order of recruitment at which each SMU appeared within a task in one block was tabulated and compared qualitatively with other blocks.

The firing rates of each identified SMU at each level of force for the two-step biting task trials were calculated as the number of times a particular SMU action potential occurred and then divided by the time of the analysis period. The data were confirmed to follow a normal distribution (Shapiro-Wilk test, $P > .05$). The main effect of repeating the jaw task was tested first with repeated-measures ANOVA with Bonferroni correction for multiple comparisons of all discriminated SMU firing rates for each jaw biting task across all of the blocks. The overall mean of the firing rates for all the SMUs was then calculated for each participant for each block in order to determine if there was an effect of block on SMU firing rates for the two-step biting tasks. Repeated-measures ANOVA with correction for multiple comparisons was used to determine differences in firing rates among the three blocks within each biting task. Statistical significance for all statistical tests was accepted at $P < .05$.

Results

Threshold Analysis of Temporalis SMU Activity During Ramp Biting Tasks

Of the 75 SMUs studied in the slow ramp and fast ramp biting tasks, 37 were able to be assessed for threshold analysis in the slow ramp biting task and 35 in the fast ramp biting task. The main effect of block (baseline, HS, IS) was not statistically significant ($P > .05$) across all threshold values for both the slow ramp biting task and the fast ramp biting task (Fig 1).

Table 1 Mean Thresholds (N) for All Repetitions for Each Block in Slow Ramp Biting Task Under Baseline (B), Hypertonic Saline (HS) Infusion, and Isotonic Saline (IS) Infusion

Participant ID	SMU	B	HS	IS	B SD	HS SD	IS SD	HS-IS
4	11	22.0	24.6	23.1	0	7.1	3.4	+
	12	29.5	27.0	23.5	5.6	6.0	0.8	+
	13	46.8	49.4	39.6	5.6	1.3	5.4	+
	14	28.5	24.8	22.3	7.3	6.7	1.7	+
	15	40.1	35.2	29.0	4.1	8.7	4.7	+
5	16	20.0	24.7	28.1	3.0	5.5	7.8	–
	17	45.2	43.0	50.5	1.7	2.8	1.9	–
	18	44.0	51.7	58.4	2.8	3.3	1.0	–
	19	49.4	42.7	48.8	2.2	1.9	5.6	–
	20	53.7	51.6	57.6	1.0	1.3	3.7	–
6	23	46.0	18.4	20.4	7.5	8.0	3.3	–
	26	57.3	44.1	41.8	6.0	4.7	1.7	+
	27	54.4	50.0	46.6	6.4	0.1	7.6	+
7	29	12.5	22.8	26.5	1.1	10.1	8.3	+
11	35	2.4	2.8	1.4	0.3	0.9	0.2	+
	36	12.6	13.1	7.7	4.9	6.6	7.2	+
	37	36.0	33.8	27.3	5.6	4.6	2.9	+
	38	33.1	35.1	30.6	4.0	5.2	3.2	+
	39	23.9	26.6	26.9	2.0	2.7	2.3	o
12	40	38.2	19.1	33.9	6.1	0.7	6.1	–
	41	38.7	26.1	39.4	5.1	1.7	4.6	–
	42	33.3	17.1	29.4	5.1	2.2	2.9	–
13	45	24.4	25.6	30.0	5.9	0.3	3.5	–
	46	27.8	30.4	34.3	4.7	4.9	5.7	–
	47	30.0	33.1	29.7	4.7	2.8	2.2	+
	48	46.0	56.0	46.8	5.1	5.9	7.8	+
15	55	20.1	23.0	24.9	2.0	3.8	1.7	–
	56	33.0	38.5	44.1	6.7	6.6	0.2	–
	57	46.4	47.8	52.1	7.3	3.0	4.8	–
	59	42.5	40.4	49.3	7.7	5.6	5.6	–
17	66	41.0	40.5	32.1	9.8	2.4	21.0	+
18	72	30.3	13.1	33.7	1.9	7.3	0.1	–
	73	31.5	16.9	41.9	4.0	6.0	6.5	–
	74	27.7	12.4	22.6	1.0	5.3	1.5	–
	75	37.2	21.0	35.2	4.8	7.2	0.0	–
19	76	10.1	14.4	8.7	1.9	3.8	0.7	+
	78	33.0	37.1	28.9	6.5	2.5	7.3	+

SMU = single-motor unit; HS–IS = individual analysis of threshold where it increased (+), decreased (–), or exhibited no change (o) during HS infusion in comparison with IS infusion. No statistical tests were used in this analysis.

The individual SMU analysis (see Methods) is shown in Tables 1 and 2 for mean thresholds recorded under baseline, HS, and IS blocks. For the slow ramp biting task (n = 37) in Table 1, 17 SMUs had a higher mean threshold during HS compared to IS, while 19 SMUs exhibited a decrease in mean

Table 2 Mean Thresholds (N) for All Repetitions for Each Block in Fast Ramp Biting Task Under Baseline (B), Hypertonic Saline (HS) Infusion, and Isotonic Saline (IS) Infusion

Participant ID	SMU	B	HS	IS	B SD	HS SD	IS SD	HS–IS
2	7	38.8	40.6	24.2	1.6	6.7	7.6	+
4	11	4.5	19.9	4.5	2.4	7.1	2.8	+
	12	27.4	31.7	22.6	7.0	2.8	6.1	+
	13	51.4	64.5	43.9	7.1	2.0	0.7	+
	14	5.7	13.2	5.3	4.2	13.5	3.7	+
	15	44.2	46.6	30.9	6.6	11.8	6.1	+
5	16	21.5	29.0	31.9	1.7	4.7	4.4	–
	17	48.6	46.2	55.2	0.2	3.8	5.5	–
	19	59.1	53.0	55.3	4.0	2.2	3.3	–
6	23	46.9	23.0	24.9	3.6	3.1	6.8	–
	26	53.6	47.3	43.0	3.4	11.8	7.3	+
7	29	7.8	17.1	30.7	0.8	0.4	2.4	–
11	35	2.3	2.9	2.6	0.4	0.5	1.4	o
	36	7.8	18.5	9.9	3.2	2.3	2.9	+
	37	30.1	33.6	18.2	5.9	7.5	5.9	+
	38	36.4	33.6	24.1	0.7	5.4	4.6	–
	39	22.0	29.9	35.9	2.8	7.6	6.4	–
12	40	32.0	24.1	35.7	7.5	0.6	2.1	–
	42	32.9	24.5	34.4	2.8	2.1	1.5	–
13	45	27.7	28.6	23.6	5.8	5.7	2.7	+
	46	35.2	29.5	25.9	6.1	7.8	3.6	+
	47	32.9	26.4	25.8	5.2	6.5	4.9	o
15	55	26.6	31.0	26.6	6.2	0.5	5.2	+
	56	42.0	32.0	42.5	4.7	5.3	0.9	–
	57	49.8	63.9	49.2	1.7	7.4	4.9	+
	59	52.0	63.2	47.1	9.1	1.6	5.6	+
17	66	58.3	35.2	49.7	0.0	6.5	6.4	–
18	72	16.4	16.9	29.3	1.9	4.5	3.6	–
	73	29.8	21.9	39.1	3.2	5.3	4.4	–
	74	19.2	15.1	17.8	6.6	0.8	2.4	–
	75	34.4	25.9	38.2	4.8	0.8	2.4	–
19	76	9.1	22.6	8.7	3.8	4.7	3.7	+
	77	21.4	20.7	15.0	6.1	3.1	3.6	+
	78	35.9	44.0	32.3	1.6	2.0	1.3	+
	80	42.4	33.7	28.9	2.7	2.6	1.1	+

SMU = single-motor unit; HS–IS = individual analysis of threshold where it increased (+), decreased (–), or exhibited no change (o) during HS infusion in comparison with IS infusion. No statistical tests were used in this analysis.

threshold and 1 showed no change. For the fast ramp biting task (n = 35; Table 2), 18 SMUs had a higher mean threshold for HS when comparing to IS, where 15 units exhibited a decrease, and 2 showed no change.

Sequence of Recruitment for Each Block During Ramp Biting Tasks

The data from 12 participants were able to be analyzed for a comparison of recruitment sequences among baseline, HS, and IS in the slow ramp biting

Table 3 Sequence of Recruitment of SMUs in the Anterior Temporalis Muscle During the Slow Ramp Biting Task

Participant ID	B	HS	IS	B × HS	B × IS	HS × IS	First solution
1	1 / 2	2 / 1	2 / 1	Different	Different	Same	HS
2	7 / 8	7 / 8	7 / 8	Same	Same	Same	IS
4	14 / 12 / 11 / 15 / 13	14 / 11 / 12 / 15 / 13	14 / 11 / 12 / 15 / 13	Different	Different	Same	IS
5	16 / 17 / 19 / 20	16 / 19 / 17 / 20	16 / 17 / 19 / 20	Different	Same	Different	HS
6	23 / 26 / 27	23 / 26 / 27	23 / 26 / 27	Same	Same	Same	IS
11	35 / 36 / 39 / 38 / 37	35 / 36 / 39 / 37 / 38	35 / 36 / 37 / 39 / 38	Different	Different	Different	HS
12	42 / 40 / 41	42 / 40 / 41	42 / 40 / 41	Same	Same	Same	IS
13	45 / 46 / 47 / 48	45 / 46 / 47 / 48	45 / 47 / 46 / 48	Same	Different	Different	HS
15	55 / 56 / 57 / 59	55 / 56 / 59 / 57	55 / 56 / 59 / 57	Different	Different	Same	HS
16	60 / 61 / 63	61 / 60 / 63	60 / 61 / 63	Different	Same	Different	HS
18	74 / 72 / 73 / 75	74 / 72 / 73 / 75	74 / 72 / 73 / 75	Same	Same	Same	HS
19	76 / 77 / 78	76 / 77 / 78	76 / 77 / 78	Same	Same	Same	IS

Each SMU is indicated by a number, and the order in which each SMU is recruited is indicated by the sequence of those numbers. Different = when at least one SMU appeared in a different order in one of those blocks in comparison with the other block being compared; Same = all SMUs appeared in the same order in both blocks; first solution = the first solution injected in that participant; B = baseline; IS = isotonic saline infusion; HS = hypertonic saline infusion.

Table 4 Sequence of Recruitment of SMUs in the Anterior Temporalis Muscle During the Fast Ramp Biting Task

ID	B	HS	IS	B × HS	B × IS	HS × IS	First solution
1	1 / 2	2 / 1	1 / 2	Different	Same	Different	HS
4	11 / 14 / 12 /15 / 13	11 / 14 / 12 /15 / 13	11 / 14 / 12 /15 / 13	Same	Same	Same	IS
5	16 / 17 / 19	16 / 17 / 19	16 / 19 / 17	Same	Different	Different	HS
6	23 / 27 / 26	23 / 26 / 27	23 / 26 / 27	Different	Different	Same	IS
7	29 / 30	29 / 30	29 / 30	Same	Same	Same	HS
11	35 / 36 / 39 / 37 / 38	35 / 36 / 38 / 39 / 37	35 / 36 / 37 / 38 / 39	Different	Different	Different	HS
12	42 / 40	40 / 42	42 / 40	Different	Same	Different	IS
13	45 / 46 / 47	45 / 46 / 47	45 / 47/ 46	Same	Different	Different	HS
15	55 / 56 / 57 / 59	55 / 56 / 59 / 57	55 / 56 / 59 / 57	Different	Different	Same	HS
16	60 / 63	60 / 63	60 / 63	Same	Same	Same	HS
18	74 / 72 / 73 / 75	74 / 72 / 73 / 75	74 / 72 / 73 / 75	Same	Same	Same	HS
19	76 / 77 / 78 / 80	77 / 76 / 80 / 78	77 / 76 / 80 / 78	Different	Different	Same	IS

Each SMU is indicated by a number, and the order in which each SMU is recruited is indicated by the sequence of those numbers. Different = when at least one SMU appeared in a different order in one of those blocks in comparison with the other block being compared; Same = all SMUs appeared in the same order in both blocks; first solution = the first solution injected in that participant; B = baseline; IS = isotonic saline infusion; HS = hypertonic saline infusion.

task (Table 3) and in the fast ramp biting task (Table 4). For the comparison between HS and IS, 4 participants had a different recruitment sequence for the slow ramp biting task (Table 3), and 5 participants had a different sequence for the fast ramp biting task (Table 4). For the slow ramp biting task, the order of recruitment was different in 4 participants, and the first solution applied was the HS. For the fast ramp task, the order of recruitment was different in 5 participants, and the HS solution was applied first in 4 of them.

Table 5 Firing Rates of SMUs for Step 1 of the Two-Step Biting Task

Participant	SMU	HS	IS	Higher firing rate	First solution
1	1	17.7	22.7	IS	HS
	2	18.8	19.2	IS	
	3	–	22.9		
	5	16.2	–		
2	7	16.0	21.1	IS	IS
5	16	8.8	6.1	HS	HS
6	26	20.1	15.5	HS	IS
	27	–	5.3		
7	29	15.9	15.8	HS	HS
	30	10.0	11.7	IS	
11	35	14.0	15.8	IS	HS
	36	19.5	18.1	HS	
13	45	9.5	10.9	IS	HS
	46	11.0	9.3	HS	
	49	12.0	9.0	HS	
15	55	15.1	19.5	IS	HS
16	60	11.0	15.0	IS	HS
	63	–	19.7		
17	69	16.0	–		IS
19	76	–	8.7		IS

Firing rates for units that were present in only one of the two blocks are highlighted in gray.
HS = hypertonic saline; IS = isotonic saline.

Table 6 Firing Rates for Step 2 of the Two-Step Biting Task

Participant	SMU	HS	IS	Higher firing rate	First solution
1	1	22.2	10.0	HS	HS
	2	19.0	11.0	HS	
	3	–	12.0		
	4	–	15.9		
	5	19.1	–		
4	13	13.5	11.4	HS	IS
10	34	18.0	18.1	IS	IS
11	37	22.0	20.3	HS	HS
	38	22.5	16.3	HS	
13	51	15.0	8.0	HS	HS
15	55	23.3	21.5	HS	HS
	56	18.3	17.3	HS	
	57	14.0	17.3	IS	
	59	23.0	18.7	HS	
19	76	15.1	17.3	IS	IS

Firing rates for units that were present in only one of the two blocks are highlighted in gray.
HS = hypertonic saline; IS = isotonic saline.

Firing Rates During Two-Step Biting Task

The data of 62 discriminated SMUs were further analyzed to calculate the firing rates during the 2-second stable period of step 1 and step 2 of the two-step biting task. Of the 62 SMUs discriminated during the two-step biting task, the firing rates of 20 SMUs were able to be characterized during the HS or IS or both blocks during step 1 of the task (Table 5), and 15 SMUs were discriminated during step 2 (Table 6). There was no significant effect on the firing rate values of repeating the task of step 1 and step 2 during each block of the two-step biting task ($P > .05$; repeated-measures ANOVA). There was no significant effect of block on mean SMU firing rates at step 1 ($P = .950$, n = 13) or at step 2 ($P = .215$, n = 8). Multiple comparisons with Bonferroni correction showed no significant differences in firing rates between blocks.

Of the 20 SMUs discriminated during step 1, 8 SMUs (SMUs 1, 2, 7, 30, 35, 45, 55, and 60) showed a decrease in firing rates in HS in comparison with IS, and 6 SMUs (SMUs 16, 26, 29, 36, 46, and 49) showed an increase in firing rates. There did not appear to be any association between whether there was a decrease in firing rate or an increase in firing rate and the sequence of infusion; that is, whether HS was performed first, or IS was performed first. Four SMUs (SMUs 3, 27, 63, and 76) were de-recruited during the HS, while 2 SMUs (SMUs 5, 69) were recruited.

For the 8 SMUs that decreased their firing rates during HS (Table 5), 2 SMUs were recorded at a slightly lower force during the HS in comparison with the IS, and 6 were recorded at a slightly higher force. For the 6 SMUs that increased their firing rates during the HS, 2 were recorded at a slightly lower force during the HS, and 4 were recorded at a slightly higher force during the HS.

Table 6 lists the firing rates of the 15 SMUs characterized during step 2 of the two-step biting task. Of these 15 SMUs, 3 (SMUs 34, 57, and 76) showed a decrease in firing rates in HS in comparison with IS, and 9 SMUs (SMUs 1, 2, 13, 37, 38, 51, 55, 56, and 59) showed an increase in firing rates. Two SMUs (SMUs 3, 4) were de-recruited during the HS, while 1 SMU (SMU 5) was recruited. For the 3 SMUs that decreased their firing rates during the HS in comparison with the IS, all 3 were recorded at a slightly higher force during the HS. For the remaining 9 SMUs that increased their firing rates during the HS, 2 were recorded at a slightly lower force during the HS in comparison with the IS, and 7 were recorded at a slightly higher force.

Discussion

The main findings of the present study are that *(1)* there was no significant main effect of block (baseline, HS infusion, IS infusion) on thresholds for the slow ramp and the fast ramp biting tasks (Fig 1); *(2)* individual SMUs could show increases, decreases, or no change in thresholds during HS compared to IS (Tables 1 and 2); *(3)* there was evidence of changes in recruitment sequences in the ramp biting task for the comparison between HS and IS (Tables 3 and 4); *(4)* there was no significant effect of block on SMU firing rates at step 1 or at step 2 ($P > .05$) and no significant differences in firing rates between blocks ($P > .05$) (Tables 5 and 6); and (5) individual SMUs could show increases, decreases, or no change in firing rates during HS compared to IS (Tables 5 and 6).

The findings of the present study mostly do not support the hypothesis. Therefore, in comparison with control, experimental noxious stimulation of the right masseter muscle does not result in significant changes in the firing rates and thresholds of SMUs of the nonpainful, synergistic right anterior temporalis muscle during a goal-directed isometric biting task performance. The absence of a main effect in the grouped data has a parallel in the recently reported data showing an absence of a significant main effect of noxious masseter stimulation on the surface-recorded EMG activity from the temporalis and masseter muscles during the same tasks.[19] While no significant main effects were noted for SMU thresholds or firing rates across the blocks, there was some evidence that individual SMUs could show increases or decreases in thresholds or firing rates, or could show changes in recruitment sequences for comparisons between the HS and IS. The absence of significant main effects may reflect the individual changes in SMU threshold and firing rates, where both increases and decreases could be observed at the same recording site. While these individual SMU effects were not identified as significant, the data are suggestive that, in addition to recruitments and de-recruitments of temporalis and masseter SMUs occurring during masseter noxious stimulation[16,24,25] and reflecting significant changes to the excitatory drive to motoneurons, more subtle changes to the thresholds, firing rates, and/or sequences of recruitment might occur for individual SMUs that remain active under both pain and control conditions. These data therefore provide some preliminary suggestive evidence that nociceptive activity may also result in more subtle changes to the excitatory drive to motoneurons. These possible, more subtle effects on the excitability of motoneurons may be a factor contributing to the reorganization of motor unit activity, as proposed in the more recent models of pain-motor interaction, the IPAM and the NTAP.[7,26] While some SMUs might show an increase in activity and others a decrease in activity even at the same site in the muscle, the recording from surface EMG activity, which records activity from many SMUs, might easily show no net change in summated activity.

Effects on SMU Thresholds in Ramp Tasks

The absence of significant main effects and interactions for the SMU thresholds in the ramp tasks is consistent with the findings of previous studies also showing no effects on thresholds of SMUs from the masseter muscle during standardized biting tasks during HS infusion[32] or capsaicin injection[29] into the masseter in comparison with control. One possible explanation for the finding that the recruitment threshold of the SMUs did not significantly decrease during painful contraction in the present study could be that the recruitment of additional SMUs was enough to maintain constant force output without changing the recruitment threshold. It has also been previously reported that changes in firing rates of some existing SMUs might also contribute,[29] although a significant main effect was not identified in the present study. Another possibility to consider, as mentioned previously, is that the excitability of individual motoneurons might be differentially affected by the nociceptive stimulus, with the result that some SMUs would show increases and others decreases in threshold. For example, a reduction in the threshold of a particular SMU would mean that force would be generated earlier from that SMU, while the thresholds of other SMUs might be increased. Future studies could address this by increasing the trial repetitions so that statistical analysis could be carried out on individual SMUs. Future studies could also consider whether pain referral patterns have an influence on any possible changes in SMU thresholds; pain referral patterns were not analyzed in the present study because only two participants reported referral to the temporalis muscle, as previously reported.[25]

SMU Recruitment Sequences During HS Infusion vs IS Infusion

Some participants exhibited recruitment sequences that remained the same under both blocks, while other participants changed their recruitment sequence.

Changes in the recruitment order have been previously reported for the masseter muscle during HS infusion in comparison with control,[24] analogous findings have also been reported in the limb literature,[8,9] and the findings from studies of neck muscle activity in pain are consistent with changes in recruitment order.[33] In the present study, where the recruitment sequence was changed, it was noted that HS was usually the first solution injected, and there may therefore be an effect on the sequence of recruitment from the order in which the solutions were applied. The reason for this is unclear, although there could possibly be some psychologic effect related to the first experience of an infusion.

Effects on SMU Firing Rates

Overall, there was no significant difference in firing rates of SMUs when comparing the HS and IS. This finding is consistent with previous findings[24] of no significant main effects for HS or IS infusions on the firing rates of SMUs in the masseter muscle during the performance of a biting task. One possible explanation for the absence of an overall effect of pain on the firing rates is that the same force is achieved by the recruitment of additional higher threshold motor units during the painful contraction while lower threshold motor units are de-recruited (or vice versa), and there was no additional need for a change in firing rates of SMUs that remain active.

A qualitative analysis of the effects on firing rates showed that some SMUs could show increases in firing rates, while other SMUs could show decreases in firing rates during HS in comparison with IS, and this finding is consistent with previous reports.[16,24] These changes in individual SMU firing rates do not appear to relate to small variations between blocks in the force levels achieved in the two-step biting tasks, but rather reflect an individual pain-related effect. Variations in the firing rates of SMUs during pain is an additional mechanism to maintain force output despite the de-recruitment or recruitment of other SMUs that were noted in previous studies.[16,24,25] Thus, for example, an increase in the firing rates of one or more SMUs might compensate for the decrease in firing rate or cessation in activity of other SMUs. However, this explanation does not appear to be consistent with the findings of a study showing that changes in firing rates of low-threshold motor units in muscles with a synergistic function to the painful muscle do not appear to account for the maintenance of force during a painful constant force contraction, as motor-unit firing rate was reduced in the synergist muscles.[34] This also suggests the effect of nociceptor stimulation is not localized and has a broad effect on synergist muscles.[34]

Findings in Relation to the IPAM and the NTAP

The findings of the present study, together with other recent data sets,[16,24,25] suggest that in the presence of noxious stimulation of one jaw muscle, there are mostly minimal or no effects on SMU activity. Some evidence has been provided that the activity of a few SMUs undergoes a reorganization in terms of recruitments and de-recruitments of SMUs, and this occurs not only in the painful muscle, but also in nonpainful muscles in the jaw motor system during task performance.[16,24,25] The present findings provide suggestive evidence that this reorganization may also extend to more subtle increases and/or decreases in firing rates, thresholds, and recruitment sequences of individual SMUs in the nonpainful synergist muscles. These new data tend to be more in line with more recent models of pain-motor interaction[7,26] rather than the earlier VCT or the PAM, which proposed uniform increases or decreases in activity throughout the motor system in the presence of pain.

Conclusions

The present study of experimental infusion of HS into the masseter muscle extends the findings from the previous accompanying study, which showed that, in comparison with control, there was no significant effect on the ability to perform the biting tasks employed and there was no effect on the occurrences of at least 70% of the SMUs within the masseter and anterior temporalis muscles during the tasks.[25] The present study demonstrates the absence of significant main effects on anterior temporalis SMU thresholds and firing rates of those SMUs remaining active during HS and IS infusions. Some data were provided to suggest that an effect on the sequence of recruitment, or the thresholds or firing rates of individual SMUs, could be altered during HS in comparison with IS. Taken together, these findings provide suggestive evidence that the reorganization of motor-unit activity that appears to occur in pain as proposed in the more recent models of pain-motor interaction[7,26,27] and which has been demonstrated in the jaw motor system through recruitments or de-recruitment of SMUs in both painful and nonpainful muscles may extend to more subtle increases and decreases in firing rates and thresholds of SMUs, as well as recruitment sequences in the nonpainful synergist muscles.

Highlights

Basic Science Research

- Experimental infusion of hypertonic saline into the masseter muscle had no significant main effects on thresholds and firing rates of single-motor units within the anterior temporalis muscle
- There was suggestive evidence that infusion of hypertonic saline into the masseter muscle could affect the sequence of recruitment or the thresholds or firing rates of individual single-motor units within the anterior temporalis muscle
- The findings provide tentative support for the conclusion of a reorganization of motor unit activity occurring not only in the painful jaw muscles but also in the nonpainful synergistic muscles.

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