Effects of Acute Experimental Stress on Pain Sensitivity and Cortisol Levels in Healthy Participants: A Randomized Crossover Pilot Study

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Aims: To investigate pain sensitivity in the masseter muscle and index finger in response to acute psychologic stress in healthy participants. **Methods:** Fifteen healthy women (23.7 ± 2.3 years) participated in two randomized sessions: in the experimental stress session, the Paced Auditory Serial Addition Task (PASAT) was used to induce acute stress, and in the control session, a control task was performed. Salivary cortisol, perceived stress levels, electrical and pressure pain thresholds (PTs), and pain tolerance levels (PTLs) were measured at baseline and after each task. Mixed-model analysis was used to test for significant interaction effects between time and session. **Results:** An interaction effect between time and session occurred for perceived stress levels ($P < .001$); perceived stress was significantly higher after the experimental task than after the control task ($P < .01$). No interaction effects occurred for salivary cortisol levels, electrical PTs, or pressure PTLs. Although significant interactions did occur for electrical PTL ($P < .05$) and pressure PT ($P < .001$), the simple effects test could not identify significant differences between sessions at any time point. **Conclusion:** The PASAT evoked significant levels of perceived stress; however, pain sensitivity to mechanical or electrical stimuli was not significantly altered in response to the stress task, and the salivary cortisol levels were not altered in response to the PASAT. These results must be interpreted with caution, and more studies with larger study samples are needed to increase the clinical relevant understanding of the pain mechanisms and psychologic stress. *J Oral Facial Pain Headache 2020; 34:281–290. doi: 10.11607/ofph.2488*

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Temporomandibular disorders (TMD) are conditions that involve the masticatory muscles and the temporomandibular joints (TMJs). Symptoms and signs associated with these conditions are pain and tenderness of the masticatory muscles and TMJs, pain during mastication, limited jaw function, and TMJ sounds.[1,2] TMD pain is more frequently reported in women and has a prevalence of 3% to 15% in the general population.[3] It is known that chronic psychologic stress could be involved in the pathophysiology of painful TMD,[4] but it is not known whether acute psychologic stress could be a potential onset factor for painful TMD and lead to increased pain sensitivity in the trigeminal region. The exact role of psychologic stress, whether acute or chronic, remains unclear in the pathophysiology of TMD pain.

Levels of psychologic stress and daytime cortisol are higher in TMD pain patients compared to healthy participants.[5–8] During stressful situations, signs and symptoms in TMD patients increase,[9] and the release of cortisol in response to acute experimental psychologic stress is significantly higher than in healthy participants.[7] One recent study showed that patients with TMD have an upregulated hypothalamic-pituitary-adrenocortical (HPA) axis, and a possible contributing mechanism for this could be psychologic factors.[10]

Exposure to acute psychologic stress activates the stress system, the main components of which are the autonomic nervous system (ANS) and the HPA axis.[11,12] The stress system is mainly regulated in the brainstem and the hypothalamus.[11,12] In response to stress, other aspects of

the central nervous system are also activated, such as the amygdala, hippocampus, and the pro-opiomelanocortin system of the hypothalamus.[11,12] Moreover, several systems that are related to stress response are involved in pain modulation.[13] Therefore, in addition to psychologic stress, a painful stimulus can also activate the HPA axis.[14] Acute psychologic stress has been associated with both analgesia and hyperalgesia.[15,16] When analgesia develops due to acute psychologic stress, both opioid and nonopioid mechanisms are thought to be implicated.[17] Activation of the HPA axis due to acute psychologic stress induces secretion of the corticotrophin-releasing hormone (CRH) from the hypothalamus, which binds to CRH receptors on the pituitary gland, leading to the release of adrenocorticotropic hormone (ACTH) to the circulatory system. ACTH interacts with the adrenal cortex, and glucocorticoid cortisol is subsequently released.[12]

The salivary cortisol level is considered to be a reliable indicator of activity in the HPA axis in response to stress[18] and is widely used as such.[19–21] Seven minutes after exposure to a stressful stimulus, higher levels of plasma cortisol can be detected.[22] Salivary cortisol levels peak approximately 1 to 2 minutes after the plasma peak.[22,23]

Several modalities can be used to induce and study acute psychologic stress; for example, the psychologic stress task the Paced Auditory Serial Addition Task (PASAT).[24] The PASAT increases heart rate and blood pressure, which activates the ANS in response to acute psychologic stress.[25,26] The PASAT also reduces experimental pain in healthy participants, most likely by activation of endogenous pain-inhibiting mechanisms.[25,26] Furthermore, it has been observed that the pain thresholds in patients with TMD pain are reduced in response to the PASAT (ie, mechanical hyperalgesia).[27] However, it is not known whether the PASAT can alter salivary cortisol levels. Although other stress tasks have demonstrated an increase in pain sensitivity,[28–30] the PASAT has not.[26]

The knowledge gap in how pain sensitivity is associated with psychologic stress in healthy participants and in patients with TMD pain is the reason for the present study. This study investigated pain sensitivity in the human masseter muscle in response to acute experimental psychologic stress induced by PASAT in healthy participants. The following hypotheses were tested: *(1)* experimental psychologic stress would lead to mechanical and electrical hyperalgesia; *(2)* the level of salivary cortisol would increase and is a reliable measure of the level of cortisol in response to experimental psychologic stress; and *(3)* experimental psychologic stress would increase heart rate and blood pressure.

Materials and Methods

Participants

Fifteen healthy women participated in the present study (mean age: 23.7 ± 2.3 years). Only five used oral contraceptives. All participants were recruited at Malmö University and were screened per the Research Diagnostic Criteria for TMD (RDC/TMD).[2] Inclusion criteria were age > 18 years; women in the beginning of the follicular phase of their menstrual cycle; healthy; and no orofacial pain complaints.

Exclusion criteria were systemic inflammatory connective tissue diseases (eg, rheumatoid arthritis); whiplash-associated disorders; widespread chronic muscle pain conditions (eg, fibromyalgia); neuropathic pain or neurologic disorders (eg, oromandibular dystonia); pain of dental origin; pregnancy or lactation; abnormal blood pressure levels; ongoing dental treatment; and/or use of analgesics (eg, paracetamol, nonsteroidal anti-inflammatory drugs [NSAIDs], salicylate drugs, opioids) or other medication that would influence pain perception (eg, anti-depressants or anti-epileptic drugs) or ANS responses 1 week before the experiment. Exclusion criteria were assessed by collection of medical histories for the participants. The participants were informed about the study and consented not to eat or drink for 1 hour before the study. They also agreed to not drink alcohol or use nicotine and to avoid excessive physical activities 12 hours prior to participation.

The Helsinki Declaration Guidelines were followed, and the Regional Ethics Review Board at Lunds University approved the study (2012/167). The participants signed an informed consent form before participation and understood that they could withdraw from the study at any time with no consequences. No financial or other compensation was given.

Study Design

The present study had a single-blinded, randomized (Random Number Generator [SPSS, IBM]) crossover design that consisted of two sessions lasting 50 minutes each: a stress session in which an experimental stress task was performed, and a control session with the inclusion of a control task. To avoid carryover effects, the interval between sessions was a minimum of 1 day. When the human body wakes from sleep, cortisol levels begin to increase and peak around 30 to 45 minutes later—this is called the cortisol awakening response.[31,32] To minimize this response and daytime effects on cortisol, each session was scheduled to begin approximately 2 hours after participants woke in the morning. At the beginning of each session, participants were asked to relax for 20 minutes. In the next 10 minutes, these baseline measurements were taken: saliva was sampled to

determine the cortisol level; perceived stress (subjective level of stress) was measured on a 0–100 visual analog scale (VAS); the electrical pain threshold (PT) and the electrical pain tolerance level (PTL) were measured on the index finger and thumb on the right hand (spinal nervous system measurements); and the pressure PT and pressure PTL were measured on the right masseter muscle (trigeminal nervous system measurements). Participants were then randomly assigned to perform either the experimental stress task or the control task; and in the next session, they performed the other task. Directly after each task, measurements of perceived stress level and of electrical and pressure PTs and PTLs were made, and new saliva samples were taken. Heart rate and systolic and diastolic blood pressure were measured before and immediately after each task (Fig 1).

One operator (A.T.), who was blinded to whether the participant in the actual session was randomized to the stress session or the control session, conducted all measurements and left the room during the tasks. All sessions were done in the same room at the Department of Orofacial Pain and Jaw Function, Faculty of Odontology, Malmö University, Malmö, Sweden, in which the ambient noise was kept low. Throughout the trial, a voice recording gave participants standardized information and instructions.

Experimental Stress Task and Control Task

The PASAT was used to induce acute experimental psychologic stress. PASAT is an audio recording that presents 251 randomized single digits (from 1 to 9) at a pace of 2.4 seconds. The participants were required to add each new digit heard to the preceding digit and then say the sum out loud. One operator assessed the participants. The duration of the experimental stress task was 10 minutes, and the maximum attainable score was 250. Participants were instructed to concentrate and to score as many correct answers as possible. Acceptable reliability for inducing experimental stress has been found for the PASAT.[25]

During the control task, the participants also listened to the PASAT audio recording, but were instructed only to repeat the digits without making any calculations.

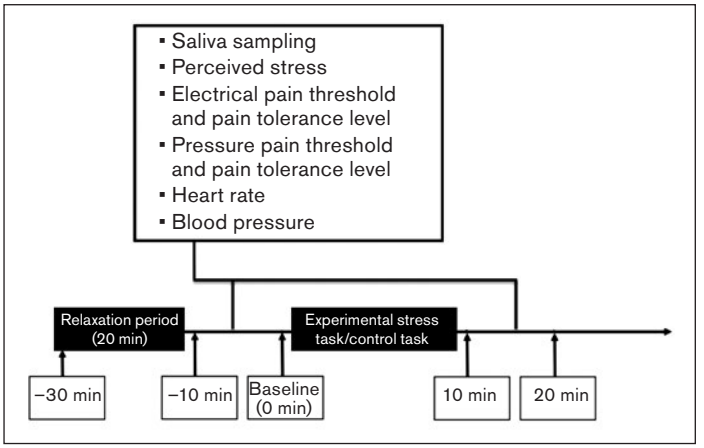

Fig 1 Schematic illustration of study design. In the first session, participants were randomly allocated to the experimental stress task or the control task. Participants performed the other task in the second session. Each session began with a 20-minute relaxation period, followed by a 10-minute period during which the saliva was sampled, perceived stress and pain threshold and pain tolerance levels for electrical and mechanical stimuli were assessed, and heart rate and blood pressure measurements were taken. This procedure was repeated immediately after each task, which lasted 10 minutes.

Saliva Sampling

Participants were asked to fast overnight and eat nothing in the morning before the study. Saliva was sampled using SalivaBio Oral Swab (SOS) (Salimetrics). Before saliva collection, the participants were asked to rinse their mouth with water. The SOS was then placed under the tongue for 3 minutes. After sampling, the swab was stored in a Salimetrics Swab Storage Tube, which was immediately frozen (–80°C). Before analysis, the saliva samples were brought to room temperature and centrifuged (1,500 g, 20°C) for 15 minutes to precipitate mucins. Salivary cortisol levels were measured using the commercially available High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit (Salimetrics) according to the manufacturer's instructions.

Assessment of Pain Thresholds and Pain Tolerance Levels

The Painmatcher (Cefar Medical) was used to assess electrical PT (the lowest electrical stimuli needed to produce a painful sensation) and electrical PTL (the lowest painful stimuli needed to produce the worst imaginable pain). The participants held the Painmatcher in their right hand between the index finger and the thumb. When turned on, the Painmatcher produced a constant low current (15 mA) with a constant frequency of 10 Hz at a random velocity. Pulse width could be increased to raise intensity, and the stimulus was stopped as soon the electrodes were released. The mean of three measurements was calculated to determine electrical PT and electrical PTL. The interval between each measurement was 30 seconds for electrical PT and 60 seconds for electrical PTL. Acceptable reliability has been reported for electrical PT and electrical PTL using the Painmatcher.[33]

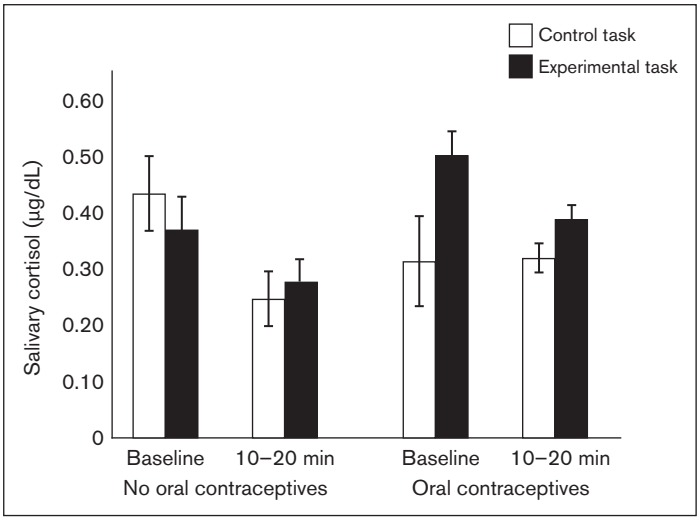

Fig 2 Mean ± standard error of the mean salivary cortisol (ug/dL) levels of participants with and without oral contraceptives, measured at baseline and after the control and experimental stress tasks.

The pressure algometer (Somedic) was used to assess pressure PT (the lowest amount of pressure in kPa needed to produce a painful sensation) and pressure PTL (the lowest painful stimuli in kPa needed to produce the worst imaginable pain). A 1-cm^2 probe was placed on the attachment at the insertion of the right masseter muscle with a constant pressure of 30 kPa/second.[34] The mean of three measurements, made at 60-second intervals, was calculated for pressure PT and pressure PTL. Acceptable reliability has been reported for pressure PT and pressure PTL measured on the masseter muscle.[35]

Assessment of Perceived Stress

Perceived stress (participant-based reports of level of stress) was assessed on a 0–100 VAS (anchor definitions: 0 = no stress and 100 = maximum imaginable stress).

Assessment of Heart Rate and Blood Pressure Levels

Heart rate and blood pressure levels were measured with an autonomic blood pressure monitor (Omron M6, Omron Healthcare). The inflatable cuff was strapped on the left upper arm at heart level for measurement. One measurement assessed the heart rate and systolic and diastolic blood pressure. Acceptable validity and reliability have been found for heart rate and blood pressure measured with this device.[36]

Statistical Analyses

All statistical analyses were conducted with SPSS for Windows, version 20. Means and SDs were calculated for age. All variables were tested for normality with Shapiro-Wilks test. Only pressure PT and PTL, heart rate, and blood pressure levels were normally distributed. After natural logarithm transformation, perceived stress levels, electrical PT and PTL, and salivary cortisol levels were also normally distributed; thus, parametric statistics could be used for all analyses.

The mixed model analysis of variance (ANOVA) for repeated measures was used to investigate the two independent groups (experimental stress task vs control task) for significant main effects of time (before and after each task) on the dependent variables. To investigate whether the dependent variable was significantly altered by the combination of factors (experimental stress task or control task; before or after the tasks), the data were analyzed for interaction effects. If an interaction effect was present (ie, the impact of one factor, such as time, depends on the level of the other factor [experimental stress task or control task]), a simple effects test adjusted for multiple comparisons was made.

Pearson correlation test with Bonferroni correction was used to analyze correlations between perceived stress levels after the stress task, electrical and pressure PTs and PTLs, and salivary cortisol levels.

Sample size was based on 5% risk of type I and 20% risk of type II errors, with an estimated ratio of differences in cortisol levels between the groups of at least 20%. The minimum sample size that was required in the present study was 15 participants; thus, 15 participants were included. All results are presented as means ± standard error of the mean (SEM) or SD. Values of $P < .05$ were considered statistically significant.

Results

Salivary Cortisol and Perceived Stress Levels

Figure 2 presents a comparison of the mean salivary cortisol levels between participants taking and not taking oral contraceptives, and Fig 3 presents the results of salivary cortisol and perceived stress levels of the entire sample. Significant main effects of time ($P < .001$) and session ($P < .050$) were observed for salivary cortisol levels, and a significant reduction occurred over time compared to baseline values. During the stress session, a significantly higher level of cortisol was found compared to the control session. No interaction effects were observed ($P > .05$).

Significant main effects on perceived stress levels were observed for time ($P < .001$) and session ($P < .050$). A significant interaction effect between time and session was also found ($P < .001$). After the task performances, a simple effect of time was

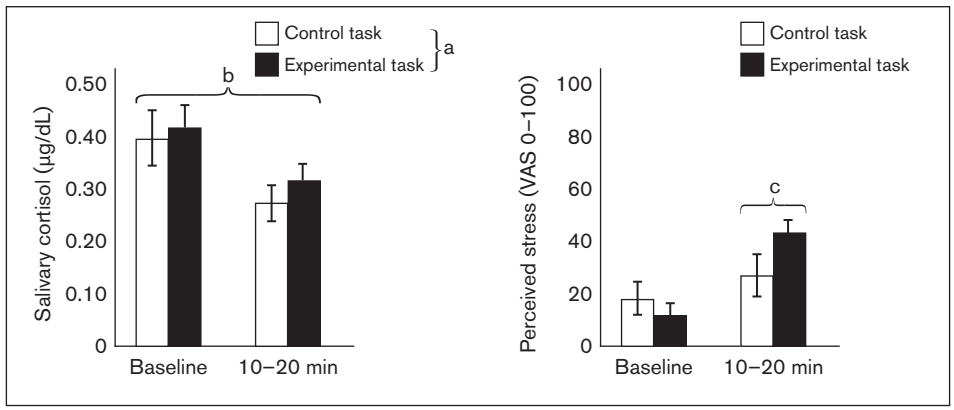

Fig 3 Mean ± standard error of the mean salivary cortisol (ug/dL) and perceived stress levels measured at baseline and after the control and experimental stress tasks. Significant differences between sessions: [a]$P < .050$. Significant change over time: [b]$P < .001$. Simple effect between time and session after the task performances: [c]$P < .01$.

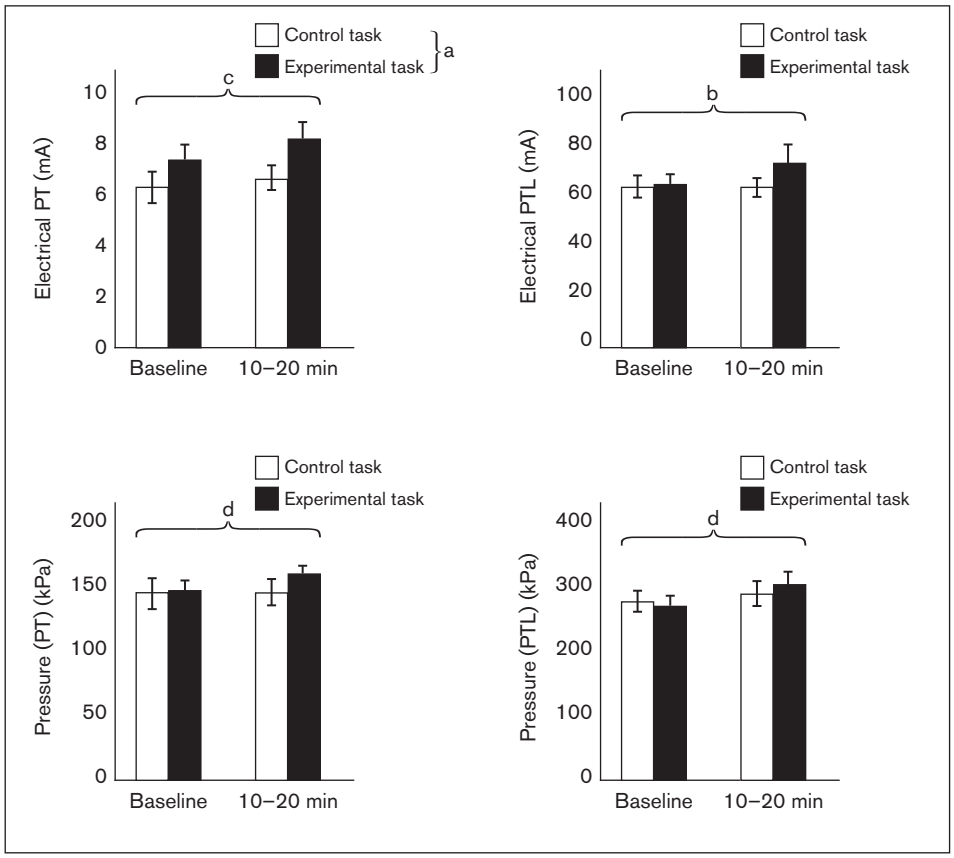

Fig 4 Mean ± standard error of the mean of electrical PT and PTL and pressure PT and PTL measured at baseline and after the control and experimental stress tasks. Significant differences between sessions: [a]$P < .050$. Significant change over time for both sessions: [b]$P < .050$, [c]$P < .010$, [d]$P < .001$.

observed between sessions ($P < .01$), with a significantly higher level of perceived stress after the experimental stress task compared to the control task.

Pain Thresholds and Pain Tolerance Levels

Figure 4 presents the results for electrical and pressure PT and PTL. There were significant effects of time ($P < .010$) and session ($P < .050$) on mean electrical PT. Electrical PT increased significantly over time, and in the stress session, a significantly higher electrical PT was found compared to the control session. No interaction effect was observed ($P > .050$).

Mean electrical PTL increased significantly over time ($P < .050$). A significant main effect of session was not observed ($P > .05$), but a significant interaction effect between time and session was detected ($P < .050$). A tendency for mean electrical PTL to increase was observed in the stress session, but the simple effects test could not identify at what time point significant differences between sessions occurred.

Mean pressure PTs were significantly higher at the end of the sessions ($P < .001$) compared to baseline values. No significant session effects were

Table 1 Systolic and Diastolic Blood Pressure (BP) Levels and Heart Rate Measured at Baseline and After 30 Minutes in the Stress and Control Sessions

	Baseline		After stress/ control session	
	Stress	Control	Stress	Control
Systolic BP (mmHg)	108 ± 1.6	109 ± 2.5	107 ± 1.7	106 ± 2.3
Diastolic BP (mmHg)	81 ± 7.3	74 ± 1.6	74 ± 1.8	75 ± 1.2
Heart rate (beats/min)	67 ± 2.1	65 ± 1.8	66 ± 2.3	63 ± 1.9

All data are reported as mean ± standard deviation.

observed ($P > .05$). A significant interaction effect between time and session was detected ($P < .001$), but the simple effects test detected no significant differences between sessions at any time point.

Significant increases over time in mean pressure PTL ($P < .001$) occurred, but no significant session differences ($P > .05$). No interaction effect was observed between time and session ($P > .05$).

Heart Rate and Blood Pressure

Table 1 presents the results for heart rate and blood pressure levels. No main effects of time, session, or interaction between time and session were observed for systolic or diastolic blood pressure levels or heart rate ($P > .05$ for all).

Correlations

After Bonferroni correction for multiple testing, no significant correlations between perceived stress levels from the experimental stress task and any of the following were identified: electrical PT and PTL, pressure PT and PTL, or salivary cortisol levels ($P > .05$ for all).

Discussion

The main findings of the present pilot study were that the PASAT *(1)* evoked significant levels of perceived stress; *(2)* did not alter salivary cortisol levels; *(3)* did not change pain sensitivity; and *(4)* did not change blood pressure levels or heart rate.

Acute stress can be associated with both hyperalgesia and analgesia.[15,16] The experimental psychologic stress task PASAT was chosen to further investigate this duality because it has previously been reported to induce acute experimental stress successfully.[25,26,37] The present findings agree with those of others,[25,26,37] since perceived stress increased over time and was significantly higher after the experimental stress task compared to the control task. Since the main stress systems in the body are the ANS and the HPA axis, and since salivary cortisol is a reliable indicator for activity in the HPA axis,[18] salivary cortisol levels, heart rate, and blood pressure would be expected to increase in response to PASAT. However, in contrast, the results indicated that the PASAT failed to activate the HPA axis and the ANS, since no effects on salivary cortisol levels, heart rate, or blood pressure were observed.

The PASAT has been widely used and is known to provoke significant levels of stress in healthy participants.[25,37,38] During the experimental stress task, the participants perform a mathematic calculation and say the result out loud to the operator. The participants are informed that the maximum attainable score is 250 and are thus aware that they are being judged during the experimental task. This could have affected the levels of anxiety and their expectations. Unfortunately, the level of anxiety and the participants' expectations were not evaluated in the present study, which is a limitation. The provoked level of stress, however, is similar to the levels reported by others.[25,37,38]

An interaction effect between time and session was not observed for salivary cortisol levels; however, a significant reduction over time occurred for both sessions, which was unexpected. After exposure to a stressful stimulus, it takes approximately 7 minutes before cortisol is detectable in plasma[22] and another 1 to 2 minutes in saliva.[22,23] Saliva was collected from the participants immediately after each task; thus, it is most unlikely that the expected salivary cortisol peak was missed.

In the present study, 10 of the 15 female participants did not use oral contraceptives. However, studies have shown that use of oral contraceptives significantly reduces the level of salivary cortisol in women[39–41] independently of the pill phase (active or inactive).[40] It is not known whether the use of oral contraceptives influenced the results of the present study, since the study sample was too small to conduct a statistical analysis regarding the use of oral contraceptives. In order to reduce the risk of this bias, it is important to address whether the participants used oral contraceptives in future studies.

The small nonhomogenous study sample might therefore have influenced the results. A larger and more homogenous study sample will be needed to better understand the influence of sex hormones on the interaction between pain sensitivity and psychologic stress and biomarkers; however, the present pilot study will indicate relatively minor effect sizes, if any.

One factor, which is a strength, is the menstrual cycle phases. Previous studies have shown that the level of salivary cortisol is significantly higher in

women in the luteal phase compared to the follicular phase.[41,42] All participants in the present study were in the beginning of the follicular phase (self-reported).

In contrast to salivary cortisol, a more precise method that would have avoided potential effects of oral contraceptive use would have been to assess plasma cortisol levels, which are unaffected by oral contraceptive use after experimental stress.[39] Unfortunately, this was not done. No further statistical analysis was made between subgroups due to the low number of participants in each subgroup (ie, 5 vs 10). Furthermore, several other factors could influence cortisol release, such as medications, pregnancy, food intake, exercise, gender differences, and mental disorders.[43] In the present study, all participants were women, were excluded from participation if they were pregnant, were instructed not to eat or drink 1 hour before participation, and were also instructed to avoid excessive physical activity 12 hours before study participation. Thus, it seems unlikely that the previously mentioned factors would have biased the results. On the other hand, psychologic health (eg, anxiety, depression, and catastrophizing) was not evaluated in the present study, which could have influenced the results.

The most likely explanation regarding reduction in salivary cortisol after both sessions is the cortisol awakening response. Upon awakening, cortisol levels begin to increase and peak around 30 to 45 minutes later.[31,44] In the present study, saliva sampling was made at least 2 hours after awakening in order to minimize the effect of the cortisol awakening response and of daytime effects on cortisol. A third explanation might be the anxiety level of the participants. It has been demonstrated that patients with high and low levels of anxiety have different cortisol responses to experimental stress. High levels of anxiety have been associated with a higher cortisol response, and low levels of anxiety with a decrease in cortisol levels.[45] Unfortunately, the present study did not evaluate anxiety levels; thus, it is unclear whether the decrease in salivary cortisol levels is a consequence of oral contraceptive use, anxiety levels, or a delayed cortisol awakening response, which is a limitation of the study.

The ANS can be activated by stress,[11,12] and activation of this system is associated with increased blood pressure and heart rate.[46,47] Recently, studies have shown that the PASAT is associated with increased heart rate and blood pressure.[25,37,48] According to the results, the PASAT provoked significant levels of perceived stress in the participants; however, it seems that the PASAT failed to activate the ANS in the present study. Heart rate and blood pressure were not significantly altered in response to the PASAT, which is contradictory to findings of other studies.[25,37,48] In the present study, heart rate and blood pressure were not measured continuously during the PASAT, which could have affected the results. Heart rate and blood pressure were assessed prior to each task and immediately after; possibly, increases in heart rate and blood pressure during the PASAT were missed. In other studies,[25,37,48] heart rate and blood pressure were measured throughout the experiment, including during the PASAT. This methodologic difference likely explains the diverging results. Another explanation might be ascribed to the duration of the PASAT. In Bendixen et al,[25] the PASAT was used for 5 minutes, while in the present study, the PASAT was used for 10 minutes. The stress effect of PASAT on heart rate and blood pressure was highest at the beginning of the experimental task, and since a 10-minute PASAT was used in the present study, it is likely that the effect had decreased to levels that were not detectable—thus, an adaptation effect cannot be excluded, which would explain why the PASAT failed to activate the ANS. The absence of increases in heart rate and blood pressure could also be a consequence of the study design, as a crossover design was used. To avoid carryover effects, the interval between sessions was a minimum of 1 day, but it cannot be excluded that this time interval was not sufficient to prevent carryover effects which, again, could have influenced the results.

Overall, the only significant finding regarding the pain-related variables is the higher electrical PT in the experimental stress session compared to the control session; thus, the electrical stimuli–induced hypoalgesia. Previous studies have suggested that psychologic stress is commonly associated with increased pain sensitivity (hyperalgesia),[28–30] while more intense stress tasks, such as electrical shocks, are more likely to evoke hypoalgesia.[49,50] The Cathcart et al study observed a significant decrease in pressure PT after exposure to a psychologic stress task,[51] which does not agree with the results of the present study. Another study observed PT and PTL to be unaltered after exposure to experimental stress.[52] A possible explanation for the results in the present study might be ascribed to factors such as age, gender, and prior experience of stressful and painful stimuli;[53] the results might also rely on whether the participants were high responders or low responders to experimental stress. In the present study, only women were included, while in the Cathcart et al study,[51] both women and men were included, and the mean age of that study sample was slightly higher. Furthermore, it has been shown that the duration of the stress task can influence the magnitude of perceived stress and thus affect nociceptive processing.[54] On the other hand, a prolonged psychologic stress task would most likely fatigue the participants and bias the results. It seems

that the type of experimental stress and its magnitude influence nociceptive transmission and self-reported measures of pain. In the present study, another possible explanation for the lack of significant alteration of pressure PT and PTL, and of electrical PTL, could be social threats. Previous studies have observed that social threats can alter pain expression and result in increased pain intensity.[55] In the experimental stress session, the participants performed a calculation task, and it cannot be excluded that social threats may have affected the results, since an operator was judging the participants in the experimental session. Furthermore, it is also possible that social threats—ie, fear of negative evaluation after the experimental task—could have influenced the level of perceived stress. Unfortunately, the present study did not take this into consideration.

To evaluate the effects of experimental stress on pain perception, electrical and mechanical stimuli were used to evaluate the spinal (index finger and thumb) and trigeminal (masseter muscle) nervous systems, respectively. A pressure algometer was used as the mechanical stimulus in order to activate C fibers,[56,57] while the electrical stimulus activated a wide range of fibers, including A-beta fibers.[58] Both methods have been demonstrated to be reliable.[35,59] Stress-induced analgesia is most likely mediated by descending pain inhibitory pathways. These pathways originate in higher brain regions such as the cortex, hypothalamus, and amygdala.[13] Neurons from these structures project to the periaqueductal gray and the rostroventral medulla, which in turn project to the dorsal horns of the spinal cord.[13] Activation of the descending inhibitory pathway inhibits peripheral nociceptive input at the dorsal horn level in the spinal cord.[53] The descending inhibitory pain pathways can also be modulated by other mechanisms, such as monoaminergic,[60] endocannabinoid,[61] and opioidergic mechanisms.[62] Furthermore, it has also been suggested that the HPA axis is involved in the mediation of stress-induced analgesia.[63]

Overall, the present results indicate that the stress task increased electrical PT significantly, while no other significant between-group differences were observed for the pain-related variables. An important question to answer is why only the electrical PT were significantly altered between groups and no other pain-related variables were altered (ie, electrical PTL, pressure PT, and pressure PTL). As previously discussed, both psychologic and cognitive factors influence the stress response and also the processing of pain.[64] It is known that emotions can modulate the pain experience; ie, enhance or reduce the pain.[65] The interaction between valence (pleasant vs unpleasant) and arousal (calm vs excited) for the stress task is important for pain modulation.[65] Unpleasant emotions in combination with anxiety (low to moderate arousal) are associated with increased pain, while unpleasant emotions in combination with fear (high arousal) lead to reduced pain.[65] The pain sensation is reduced as a consequence of pleasant emotions in combination with minimal arousal.[65] Electrical PT was significantly higher after the stress task compared to baseline values. It cannot be excluded that the participants had a greater negative expectation (unpleasant) and high arousal (fear) after the first assessment with the electrical stimuli, which in turn could have increased the electrical PT. In one study, fear was induced in a study sample, and as a consequence of this, the participants' (all women) pain thresholds for heat were increased.[66] Another study showed that induced anxiety increases pain thresholds in women.[67] On the other hand, the pressure PT in the present study was not altered in response to the stress task, implying other explanations for the results regarding electrical PT. Lund et al observed that the electrical PT measured with the electrical stimulus was significantly increased in healthy women after 10 minutes of high-frequency transcutaneous electrical nerve stimulation.[68] In this pilot study, prior to the control or experimental task, the PTs and PTLs were assessed. One hypothesis is that the three measurements of the electrical PTL before the control/experimental stress task activated endogenous pain inhibitory mechanisms. This potential activation of the endogenous pain inhibitory system in combination with the effect of the stress response might explain the increased electrical PT observed after the experimental task.

The relationship between acute experimental stress and modulation of pain transmission is still controversial, and further research is warranted to better elucidate the association between acute stress and nociceptive processing.

A strength of the present study, which evaluated whether an analgesic effect was developed after experimental stress, was that psychophysical measurements were made both in the spinal and trigeminal nervous systems. Another advantage is that this study used a randomized, single-blinded, crossover design. One limitation, however, is that the use of oral contraceptives was not an exclusion criterion even though it has been observed that oral contraceptives influence the level of salivary cortisol.[39] Additionally, it seems that the PASAT failed to activate the HPA axis and the ANS; however, it must be emphasized that significant levels of perceived stress were provoked.

In summary, this pilot study has a few limitations: the level of anxiety, depression, catastrophizing, and expectation were not evaluated during the trial; the study sample was small and not homogenous (in terms of oral contraceptive use); and the cortisol

awakening response could have influenced the study outcome. The measurement of salivary cortisol is a very sensitive technique that is influenced by several factors, and a more precise method to use would have been to assess the cortisol levels in plasma.

Conclusions

The PASAT provoked significant levels of stress without affecting salivary cortisol levels; furthermore, the ANS was not activated, nor was nociceptive transmission altered in response to the stress task. More well-designed studies with larger sample sizes are required to investigate the association between psychologic stress and nociceptive processing in healthy participants and in patients with persistent pain conditions, such as TMD pain.

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