UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE BAURU

DYNA MARA ARAÚJO OLIVEIRA FERREIRA

Evaluation of the influence of experimental psychological stress on the quantitative sensory testing response in temporomandibular disorders patients

Avaliação da influência de estresse psicológico experimental na resposta de testes sensoriais quantitativos em pacientes com disfunção temporomandibular

BAURU
2017

DYNA MARA ARAÚJO OLIVEIRA FERREIRA

Evaluation of the influence of experimental psychological stress on the quantitative sensory testing response in temporomandibular disorders patients

Avaliação da influência de estresse psicológico experimental na resposta de testes sensoriais quantitativos em pacientes com disfunção temporomandibular

Dissertação constituída por artigo apresentada a Faculdade de Odontologia de Bauru da Universidade de São Paulo para obtenção do título de Mestre em Ciências no Programa de Ciências Odontológicas Aplicadas, na área de concentração Reabilitação Oral.

Orientador: Prof. Dr. Paulo César Rodrigues Conti

BAURU

2017

F413e

Ferreira, Dyna Mara Araújo Oliveira

Evaluation of the influence of experimental psychological stress on the quantitative sensory testing response in temporomandibular disorders patients / Dyna Mara Araújo Oliveira Ferreira – Bauru, 2017.

76 p. : il. ; 30 cm.

Dissertação (Mestrado) – Faculdade de Odontologia de Bauru. Universidade de São Paulo

Orientador: Prof. Dr. Paulo César Rodrigues Conti

Comitê de Ética da FOB-USP
Protocolo n°: 1.235.555
Data: 18/09/2015

FOLHA DE APROVAÇÃO

DEDICATÓRIA

Ao criador de todas as coisas, ***Deus****. Por segurar em suas mãos minhas preocupações e problemas, por me guiar sabiamente por caminhos que meus olhos não podem ver e por receber com amor a minha vida.*

Aos meus pais ***Dinorá Araújo Oliveira Ferreira*** *e* ***Francisco de Assis da Costa Ferreira****, por me amarem incondicionalmente, por compreenderem os momentos de minha ausência dedicados ao estudo e por sempre me fazer entender qve o futuro é feito a partir da constante dedicação ao presente.*

À minha querida irmã, ***Maria Elizabeth Oliveira Ferreira****. Metade de mim, logo, sempre ao meu lado. Por seu carinho, apoio e amizade. Podemos discutir por qualquer motivo, mas nosso amor é indiscutível.*

AGRADECIMENTOS

Em especial ao meu orientador ***Prof. Dr. Paulo César Rodrigues Conti****, pelo qual tenho profunda admiração e respeito. Obrigada professor por ter me recebido no Mestrado e por acreditar no meu potencial. Agradeço seus ensinamentos, paciência do início ao fim, oportunidades, respeito e ética. Expresso aqui minha sincera gratidão por conviver com alguém de tamanho exemplo profissional.*

Ao ***Prof. Dr. Leonardo Rigold Bonjardim****, por compartilhar ensinamentos que permitiram meu amadurecimento profissional e pessoal. Muito obrigada também pela ajuda na elaboração deste trabalho e pela concessão do Laboratório de Fisiologia Experimental para a realização da pesquisa. Por último e não menos importante, obrigada por sua paciência.*

Ao nosso grupo de pesquisa: ***Yuri Martins Costa, Juliana Stuginsk Barbosa, Henrique Müller de Quevedo, Leticia Soares Fiedler, Laila Aguiar Machado, Everardo Napoleão S. de A. Junior e Cesar Waisberg.*** *Estivemos unidos, dividimos responsabilidades, enfrentamos dificuldades e ajudamos uns aos outros. Nossa amizade vai além do ambiente de pesquisa, é muito mais que alguns testes quantitativos sensoriais. Obrigada por estarem comigo durante essa caminha e, portanto nada mais justo que dividir com vocês as congratulações por este trabalho concluído.*

Aos professores do Departamento de Prótese e Periodontia, em especial aos professores ***Dr. Luiz Fernando Pegoraro, Dr. Accácio Lins do Vale, Dr. José Henrique Rubo, Dra Ana Lúcia Pompéia Fraga de Almeida, Dr. Estevam Augusto Bonfante, Dra. Karen Hermana Neppelenbroek e Dr. Vinícius Carvalho Porto****, pela grande contribuição para minha formação profissional e pessoal durante o curso de Mestrado. Agradecimentos infinitos por todos os ensinamentos e convívio durante as clínicas e seminários.*

À ***Faculdade de Odontologia de Bauru****, Universidade de São Paulo, na pessoa da sua Diretora, Profª Drª Maria Aparecida de Andrade Moreira Machado. Sou muito grata à FOB, que considero como minha segunda casa, pela formação sólida que me proporcionou.*

Aos ***funcionários do Departamento de Prótese e Periodontia****. Muito obrigada pela prontidão e eficiência para que cada etapa do mestrado fosse cumprida adequadamente.*

Aos ***funcionários do Departamento de Ciências Biológicas****, por toda a paciência, dedicação e colaboração para que as atividades e os atendimentos da pesquisa fossem realizados a contento.*

Às ***funcionárias da clínica da pós-graduação,*** *Cleusa Gonçalves Leite e Hebe Joselina de Freitas Pereira. Obrigada pela disponibilidade, paciência e organização durante as clínicas de pós-graduação.*

Às amigas, ***Natália Almeida Bastos, Ana Paula Chappuis, Lígia Bueno, Samira Strelhow e Cindy Garcia****, que tive a felicidade de conhecer em Bauru. Sou muito grata pelo companheirismo, incentivo, ombro amigo, pelas palavras e pelo silêncio. Com vocês, as pausas entre uma atividade e outra da pós-graduação refrescava o meu humor.*

A todos os meus ***colegas de pós-graduação****, pelo desafio de aprender a conviver com diferenças. Acredito que isto sempre nos permite crescer.*

A todos aqueles qve, de alguma forma, participaram direta ou indiretamente do desenvolvimento deste trabalho.

"Ninguém ignora tudo. Ninguém sabe tudo. Todos nós sabemos alguma coisa. Todos nós ignoramos alguma coisa. Por isso aprendemos sempre."

Paulo Freire

Abstract

ABSTRACT

Evaluation of the influence of experimental psychological stress on the quantitative sensory testing response in temporomandibular disorders patients

Background: Quantitative sensory testing (QST) is a promising method for assessing the mechanisms that contribute to the development and maintenance of painful Temporomandibular Disorders (TMD). All QST responses rely on the participant´s perception; therefore a number of cognitive and psychological factors are known to directly influence results, including psychological stress. **Aims:** To assess the effects of experimental psychological stress on QST response in TMD patients and healthy volunteers. **Methods:** 20 women with myofascial TMD and 20 healthy women underwent a standardized QST protocol, including cold detection threshold (CDT), warm detection threshold (WDT), cold pain threshold (CPT), heat pain threshold (HPT), mechanical pain threshold (MPT), pressure pain threshold (PPT) and wind up ratio (WUR) at the masseter muscle. QST was conducted before and after to the Paced Auditory Serial Addition Task (PASAT), inducing acute psychological stress. ANOVA with repeated measures was performed to assess the effect of group and time on the reported stress and absolute values of QST. The significance level was set at 5% ($p=0.050$). Furthermore, Z-score profiles were generated. **Results:** The PASAT induced a significant stress reaction ($p<0.001$). After exposure to experimental stress, both healthy volunteers and TMD patients showed increase in thermal detection threshold (CDT: $F=4.25$, $p=0.017$ and WDT: $F=4.10$, $p=0.020$) and decrease in thermal pain threshold (CPT: $F=11.2$, $p<0.001$ and HPT: $F=8.13$, $p<0.001$) when compared to baseline. However, stress did not induce significant changes in MPT, PPT or WUR in both groups ($p>0.050$). **Conclusion:** The experimental psychological stress induces thermal hypoesthesia and thermal hyperalgesia on masticatory muscle, regardless of the presence of TMD painful. Overall, these findings emphasize the importance of considering the psychological stress when judging QST findings.

Key words: Pain Threshold. Stress, Psychological. Temporomandibular Joint Dysfunction Syndrome

RESUMO

RESUMO

Avaliação da influência de estresse psicológico experimental na resposta de testes sensoriais quantitativos em pacientes com disfunção temporomandibular

Contextualização: Teste sensorial quantitativo (QST) é um método promissor para avaliar os mecanismos que contribuem para o desenvolvimento e manutenção das Disfunções Temporomandibulares (DTM) dolorosas. As respostas de QST dependem da percepção do participante; portanto, uma série de fatores cognitivos e psicológicos, como o estresse, podem influenciar os resultados. **Objetivo:** Avaliar a influência do estresse psicológico experimental na resposta de QST em pacientes com DTM e voluntários saudáveis. **Métodos:** 20 mulheres com DTM (Dor Miofascial) e 20 mulheres saudáveis foram submetidas a um protocolo padronizado de QST, incluindo limiar de detecção ao frio (CDT), limiar de detecção ao calor (WDT), limiar de dor ao frio (CPT), limiar de dor ao calor (HPT), limiar de dor mecânica (MPT), limiar de dor a pressão (PPT) e somação temporal de dor (WUR) na região de masseter. QST foi realizado antes e após teste de estresse psicológico laboratorial denominado Paced Auditory Serial Addition Task (PASAT). ANOVA foi realizada para avaliar o efeito de grupo e tempo sobre o estresse relatado e valores de QST a um nível de significância de 5% ($p = 0,050$). Ademais, perfis Z-score foram gerados. **Resultados:** PASAT induziu aumento significativo no relato de estresse ($p < 0,001$). Após exposição ao estresse experimental, ambos os grupos apresentaram aumento nos limiares de detecção térmicos (CDT: $F = 4,25$, $p = 0,017$ e WDT: $F = 4,10$, $p = 0,020$) e redução dos limiares de dor térmicos (CPT: $F= 11,2$, $p < 0,001$ e HPT: $F = 8,13$, $p < 0,001$) quando comparados com valores iniciais. Entretanto, o estresse não induziu mudanças significativas em MPT, PPT ou WUR ($p > 0,050$). **Conclusão:** O estresse psicológico experimental induz hipoestesia térmica e hiperalgesia térmica na musculatura mastigatória independente da presença de DTM dolorosa. Esses achados enfatizam a importância de considerar o estresse psicológico do particpante ao interpretar os resultados de QST.

Palavras chave: Limiar de Dor. Estresse Psicológico. Síndrome da Disfunção da Articulação Temporomandibular.

TABLE OF CONTENTS

1 INTRODUCTION

1 INTRODUCTION

Temporomandibular Disorders (TMD) embraces a group of musculoskeletal conditions that involve the temporomandibular joint, the masticatory muscles, and all associated structures[1]. It is a significant public health problem affecting approximately 12% of the global population and represents the most common chronic orofacial pain condition. In addition, it is the second musculoskeletal condition (after chronic low back pain) leading to pain and disability[2]. TMD affects the individual suffering from pain but also their families and society resulting in high cost of health care and lost productivity[3].

Clinical outcomes for TMD, as other chronic pain conditions, remain disappointingly poor, because the majority of pain treatments produce clinically meaningful improvements only in a minority of the patients that receive them[4]. Many authors have suggested that one factor contributing to this state of affairs is the prevailing approach to pain classification[5-8]. Presently, pain diagnosis is based primarily on signs and symptoms, sometimes combined with evidence of disease, structural damage or injury. However, the clinical diagnosis typically provides limited information regarding the pathophysiological mechanisms underlying the pain experience that may guide choice of treatment. Because treatments exert their clinical benefits by impacting the mechanisms underlying pain, an important goal for enhancing pain care is to incorporate assessment of pain mechanisms into the patient evaluation[8,9]. One potentially promising method for assessing the mechanisms that contribute to the development and/or maintenance of chronic pain is the quantitative sensory testing (QST)[9,10,11].

QST is a psychophysical method used to assess somatosensory function, including pain perception, in individual. It is based on measurements of individual´s responses to calibrated, graded innocuous or noxious stimuli. QST uses different assessments, including thermal (thermotest), static mechanical (von Frey or Semmes Weinstein monofilaments, pressure algometer, calibrated pins), dynamic mechanical (standardized brush), and vibratory (vibrometer, graded tuning fork) stimuli. Furthermore, it has been used for testing cutaneous sensations as well as perception from deep tissue (ligaments, fasciae, muscles) and viscera[12,13]. The basic premise of QST is that physical stimuli applied to the body under normal physiologic circumstances are transduced by activating specific sets of receptors, which generate physiologic signals in specific anatomic components of the sensory nervous

system[14]. Such components include peripheral nerve fibers of specific size, which have unique physiologic properties, including fast and slow conduction velocities, as well as central pathways from the dorsal horn of the spinal cord to thalamus and to the cortical structures relevant to sensory perception and assignment of emotional attributes when dealing with noxious stimuli (Table 1, Annex A). All these contribute to perception and ultimately to the report by the participant about the physical properties of the stimulus[11,15]. QST quantifies and monitors the presence and severity of negative sensory phenomena (loss of function – i.e, hypoesthesia and hypoalgesia) and positive sensory phenomena (gain of function – i.e, allodynia and hyperalgesia).This contribute to characterize various somatosensory profiles and their neurobiological underlie mechanisms[11].

There are a great variety of proposed quantitative testing procedures, each of which allows quantifying one particular aspect of sensory function and using different methods. In order in standardize such testing, the German Research Network on Neuropathic Pain (DFNS) has developed and validated a comprehensive QST battery which allows profiling of somatosensory function[12,16]. The design of the DFNS QST battery assembles a comprehensive list of robust and validated short form tests representing measures of all relevant sub modalities of the somatosensory system. The entire battery consists of seven tests measuring 13 parameters[12,16] :

- cold (CDT) and warm (WDT) detection thresholds
- number of paradoxical heat sensations during the thermal sensory limen procedure (PHS)
- cold (CPT) and heat (HPT) pain thresholds
- mechanical detection threshold (MDT)
- mechanical pain threshold for pinprick stimuli (MPT)
- mechanical pain sensitivity (MPS)
- stimulus/response-functions for pinprick sensitivity
- dynamic mechanical allodynia (ALL)
- temporal pain summation (wind-up ratio,WUR)
- vibration detection threshold (VDT)
- pressure pain threshold (PPT)

QST has been used for decades in the research setting, particularly for diagnosing, assessing, and monitoring neuropathies[10-12] and more recently has become a promising method for assessing musculoskeletal chronic pain, including TMD[17]. Recent studies utilizing

the DFNS standardized QST protocol has demonstrated high prevalence of somatosensory abnormalities in TMD patients when compared with reference population. 82,5% – 85% of TMD patients presented somatosensory abnormalities at the most painful site, that is masticatory muscles and temporomandibular joint[18,19]. In addition, somatosensory abnormalities outside the trigeminal area (ie, hand) were found in 60% of the patients[19]. The most frequent somatosensory abnormalities were gain of function (hyperalgesia) to pressure, pinprick, cold and heat stimuli, besides increased wind-up phenomenon. Less frequently, somatosensory loss of function to detect sub modalities, such as mechanical, vibration, cold and heat detection stimuli was found[18,19]. Yang et al, using the LossGain coding system, found L0G2 (no somatosensory loss with gain of mechanical somatosensory function) was the most frequent coding in the TMD group[19]. Another study identified two subgroups of myofascial TMD patients based on their tender point scores that correlated with different QST profiles. An insensitive TMD patients subgroup resembling healthy control in tender point number and presenting more local changes (trigeminal area) in QST parameters. In contrast, sensitive TMD patients showed higher tender points scores and generalized increased pain sensitivity for the same stimuli as fibromyalgia patients, namely cold and pinprick hyperalgesia, hyperalgesia to blunt pressure and the occurrence of dynamical mechanical allodynia; suggesting an overlap in pathophysiology with fibromyalgia[20].

Increased pain sensitivity in the primary area of pain (local pain) is considered a sign of predominantly peripheral pain sensitization, whereas pain sensitivity in areas anatomically remote from the primary area of pain is thought to reflect a more central phenomenon[21-23]. In addition, the temporal summation of pain, measured by the "wind-up ratio (WUR), that is responses to repeated noxious stimuli, is considered a reflection of "wind up" and represents a psychophysical measure of central sensitization[23-25]. Deficiency in pain inhibitory mechanisms could also explain the generalized reduced pain thresholds in the TMD patients[26,27].

Taken together, the observation that different patients suffering from the same clinical disorder, i.e. TMD, may have different phenotypes of somatosensory abnormalities, suggesting different pathophysiological mechanisms underlying the pain experience, may support the concept of pain mechanisms-based personalized management.

In light of the accumulating evidence attesting the relevance of standardized QST procedures for phenotyping TMD pain patients, it is pertinent to assess factors that can influence the test itself. As all psychophysical tests, QST depends on the participant's report and cooperation, it is an expression of the participant's perception and is not an ''objective''

measurement. Therefore, a number of factors such as attention, cooperation, motivation and emotions are known to influence results[15] . Most published QST research has concentrated on standardized protocols[12,16] and test reliability[28-30] to minimize sources of results variability from methodological and environmental influences. In contrast, psychological factors such as stress, which have a critical influence on a participant's responses[31,32] and consequently on the results, have not been sufficiently studied.

Stress was originally defined by Selye as the effect of any noxious stimulus that seriously threatens homeostasis[33]. Later, the concept was refined by distinguishing between 'stressor' and 'stress response'. A stressor is considered a stimulus that threatens homeostasis and the stress response is the reaction of the organism aimed to regain homeostasis[34]. Since the first definition, the concept of stress has undergone constant evolution. Stress should be considered as a cognitive perception of uncontrollability and/or unpredictability, expressed in a physiological and behavioral response. Moreover, the reverse is not always true: the physiological response by itself does not necessarily always indicate a state of stress. Therefore, stress is primarily a perceived phenomenon and a subjective evaluation is pertinent to ascertain a stress response. Furthermore, the absence or presence of any of these responses does not include or preclude the identification of a stressful state[35].

Following the perception of an acute stressful event, there is a cascade of changes in the nervous, cardiovascular, endocrine, and immune systems. The Sympathetic Nervous System (SNS) and hypothalamic-pituitary adrenocortical (HPA) axes produce stress hormones. The SNS stimulates the adrenal medulla to produce catecholamines (epinephrine, norepinephrine). In parallel, the hypothalamus produces corticotropin releasing factor, which in turn stimulates the pituitary to produce adrenocorticotropin. Adrenocorticotropin then stimulates the adrenal cortex to secrete cortisol. Together, catecholamines and cortisol increase available sources of energy by promoting lipolysis and the conversion of glycogen into glucose. Energy is then distributed to the organs that need it most (skeletal muscles and brain) by increasing blood pressure levels and contracting certain blood vessels while dilating others. Finally, in addition to the increased availability and redistribution of energy, the acute stress response includes activation of the immune system. Macrophages and natural killer cells, the first line of defense, depart from lymphatic tissue and spleen and enter the bloodstream, temporarily raising the number of immune cells in circulation. From there, the immune cells migrate into tissues that are most likely to suffer damage during physical confrontation (i.e., the skin). Once at "battle stations," these cells are in position to contain microbes that may enter the body through wounds and thereby facilitate healing[36]. These

changes constitute the acute stress response and are generally adaptive, at least in the short term. The stress response can become maladaptive if it is repeatedly or continuously activated, such as in chronic stress situations[36].

It well known that psychological stress can influence the onset and course of the TMD painful. Several psychological factors associated with experimental pain sensitivity, as stress, predicted new onset TMD pain[37,38]. Related findings are that psychological stress is associated with greater severity and persistence of TMD-related clinical symptoms[39,40]. To our knowledge, there are no studies that evaluate the psychological stress impact on somatosensory profile of TMD patients using a standardized QST protocol.

Based on that, the aim of this study was to investigate the influence of experimental psychological stress on QST response in TMD patients and healthy volunteers using a standardized QST protocol. We hypothesized a priori that psychological stress would affect the QST response and that TMD patients would be differently affected when compared to healthy volunteers.

2 ARTICLE

2 ARTICLE

The article presented in this Thesis was written according to Journal of Oral & Facial Pain and Headache instructions and guidelines (Annex B).

INFLUENCE OF EXPERIMENTAL PSYCHOLOGICAL STRESS ON QUANTITATIVE SENSORY TESTING RESPONSE IN TEMPOROMANDIBULAR DISORDERS PATIENTS

ABSTRACT

Aims: To assess the effects of experimental psychological stress on quantitative sensory testing (QST) response in Temporomandibular Disorder (TMD) patients and healthy volunteers. **Methods:** 20 women with myofascial TMD and 20 healthy women underwent a standardized QST protocol, including cold detection threshold (CDT), warm detection threshold (WDT), cold pain threshold (CPT), heat pain threshold (HPT), mechanical pain threshold (MPT), pressure pain threshold (PPT) and wind up ratio (WUR) at the masseter muscle. The QST was conducted before and after to the Paced Auditory Serial Addition Task (PASAT), inducing acute psychological stress. Stress levels were evaluated using perceived rating of stress. ANOVA with repeated measures was performed to assess the effect of group and time on the reported stress and absolute values of QST and the significance level was set at 5% ($p=0.050$). Furthermore, Z-score profiles were generated. **Results:** The PASAT induced a significant stress reaction ($p<0.001$). After exposure to an experimental stress, both healthy volunteers and TMD patients showed increase in thermal detection threshold (CDT: $F=4.25$, $p=0.017$ and WDT: $F=4.10$, $p=0.020$) and decrease in thermal pain threshold (CPT: $F=11.2$, $p<0.001$ and HPT: $F=8.13$, $p<0.001$) when compared to baseline. However, stress did not induce significant changes in MPT, PPT or WUR in both groups ($p>0.050$). **Conclusion:** The experimental psychological stress induces thermal hypoesthesia and thermal hyperalgesia on masticatory muscle, regardless of the presence of TMD painful. Overall, these findings emphasize the importance of considering the psychological stress when judging QST findings.

Keywords: quantitative sensory testing, pain sensitivity, psychological stress, temporomandibular disorders, myofascial pain

INTRODUCTION

Temporomandibular Disorders (TMD) are a group of musculoskeletal conditions that involve the temporomandibular joint, the masticatory muscles, and all associated structures[1]. It is a significant health care problem affecting approximately 12% of the global population and represents the most common chronic orofacial pain condition[2]. TMD affects the individual suffering from pain but also their families and society resulting in high cost of health care and lost productivity[3]. The impact and prevalence of TMD are evidence of limitations in diagnostic and managing of this condition. To date, the pathophysiology and underlying pain mechanisms of TMD remain poorly understood[4,5], although evidences shows that somatosensory disturbances can be play an important role.

Quantitative sensory tests (QST) are psychophysical tests used to investigate the somatosensory function and have become a promising method for assessing musculoskeletal disorders, including TMD painful[6,7]. A comprehensive standardized QST protocol has been presented by the German Research Network on Neuropathic Pain (DFNS) where somatosensory profiles and LossGain codind system are used to understanding of mechanisms that contribute to the development and maintenance of painful states[8,9]. Utilizing the standardized QST protocol, somatosensory abnormalities were found within and outside trigeminal area in 82% and 60% of TMD patients, respectively[10,11]. The most frequent somatosensory abnormalities were gain of function (hyperalgesia) to pressure, pinprick, cold and heat stimuli, besides increased wind-up. Somatosensory loss of function to detection sub modalities, such as mechanical, vibration, cold and heat detection stimuli also were found[10,11]. Another study identified two subgroups of myofascial TMD pain based in their tender point scores that correlated with different QST profiles. An insensitive TMD subgroup resembling healthy control in tender point number and presenting more local changes (trigeminal area) in

QST parameters, while the sensitive TMD subgroup showed higher tender points scores and generalized increased pain sensitivity over the cheek, trapezius and hand, suggesting an overlap in pathophysiology with fibromyalgia[12].

In light of the accumulating evidence attesting the relevance of standardized QST procedures for phenotyping TMD patients, it is pertinent to assess factors that can influence the test itself. All QST responses rely on the participant's perception, therefore a number of factors such as attention, cooperation, motivation and emotions are known to influence results[13]. Most published QST research has concentrated on standardized protocols[8,9] and test reliability[14-16] to minimize sources of results variability from methodological and environmental influences. In contrast, psychological factors as stress, which have a critical influence on a participant's responses[17,18] and consequently on the results of testing, have not been sufficiently studied. Based on that, the aim of this study was to investigate the effects of experimental psychological stress on QST response in TMD patients and healthy volunteers using a standardized QST protocol[8,9]. We hypothesized a priori that psychological stress would affect the QST response and that TMD patients would be differently affected when compared to healthy volunteers.

MATERIAL AND METHODS

Ethics statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Human Research Ethics Committee of the Bauru School of Dentistry, University of Sao Paulo. All participants gave informed consent in writing.

Participants

The study was conducted at the Bauru School of Dentistry from August 2016 to February 2017. A total of 20 healthy female and 20 female with TMD, between 18 – 50 years of age, were recruited for the study from the local community through advertisements. We included only female subjects in order to avoid the confounding effect of sex, as sex may affect the mode with which subjects respond to pain and to stress[19].

Healthy subjects were free of any complaint or pain syndrome at the time of study participation. Patients with TMD met the criteria for myofascial pain with or without jaw opening limitation (Ia and Ib) according to the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD)[20], with pain duration of at least 3 months.

Exclusion criteria for all subjects were: pregnancy, present or previous pathology or any other skin lesions in the face (testing site), diseases causing potential neural damage (e.g, diabetes), systemic illness (metabolic, cardiovascular, or inflammatory disorders), mental illness (e.g, anxiety disorders, depression, bipolar disorder), use of medications as such muscle relaxants, anticonvulsants, antidepressants and anxiolytics. In addition, patients with TMD management performed in the last 3 months and TMD participants suffering from other causes of orofacial pain (caries, periodontal disease or atypical odontalgia) or other chronic pain (fibromyalgia, chronic widespread pain, chronic fatigue syndrome and irritable bowel syndrome) were also excluded. Furthermore, all participants were asked to not take any analgesic medication 48 hours prior to study participation.

Psychological assessment

Prior to the start of the study, measures of the degree to which a situation is appraised as stressful, state and trait anxiety, the dimension of negative thoughts and feelings related to pain were assessed in each participant with the Brazilian versions of the Perceived Stress Scale[21], State-Trait Anxiety Inventory[22] and Pain Catastrophizing Scale[23], respectively.

Stress assessment

Psychological stress was evaluated, in both groups, using a Visual Analogue Scale (VAS). The VAS consisted of a 10-cm line with 2 anchor points at its extremes, set as 0 = ‘‘no stress’’ and 10 = ‘‘worst imaginable stress”. The participants were requested to make a vertical mark on the line at the point that they felt represented their perception of their current state of stress.

Quantitative Sensory Testing

Cold detection threshold (CDT), warm detection threshold (WDT), cold pain threshold (CPT), heat pain threshold (HPT), mechanical pain threshold for pinprick stimulus (MPT), pain temporal summation (wind-up ratio, WUR) and pressure pain threshold (PPT) in accordance with the DFNS standardized protocol[8,9] at the masseter muscle were performed. The dominant side of masseter muscle body in the control group and the most painful site of TMD patients were used as reference for the test.

Thermal detection thresholds and thermal pain thresholds

Thermal sensation tests were performed using TSA 2001-II (Medoc, Israel) thermal sensory testing device. The contact area of the thermode was 16 × 16 mm. First, CDT and WDT were measured and then CPT and HPT were determined. The thermode baseline temperature was 32 °C and heated-up or cooled-down at a rate of 1 °C/s to the upper limits of 50 °C or lower limits of 0 °C. The participants were instructed to press a button on the computer mouse as soon as they perceived the respective thermal sensation of cold, warm, cold pain, or heat pain following the instructions. The procedure then ended, and the temperature returned to baseline at a rate of 1 °C/s. The mean threshold temperature was determined from three consecutive

measurements. During the experiment, the subjects were instructed not to watch the computer screen[8,9].

Mechanical pain threshold (MPT)

MPT was measured using a standardized set of Semmes–Weinstein monofilaments (Touch-Test TM Sensory Evaluators; North Coast Medical Inc., Gilroy, CA, USA) that exert forces between 0.008 g mm $^{-2}$ and 300 g mm $^{-2}$. Tests were made with the stimulator in a vertical and perpendicular position to the site of examination, and applied at a rate of 2 s on, 2 s off in ascending order. Participants were instructed to report the first perception of sharpness/pinprick. Acceptable values for the test–retest reliability for this mechanical somatosensory assessment have been demonstrated[24]. The 'method of limits' was used to determine the threshold when a series of ascending and descending stimuli intensities were applied yielding five suprathreshold and five subthreshold reports. The final threshold was the geometric mean of these measurements[8,9].

Wind-up ratio (WUR)

The WUR was measured with the same set of Semmes–Weinstein monofilaments as for MPT. The instrument that delivered a force which the participant perceived as 'slightly painful' was chosen for this test; that is, if the participant's numerical response was higher than 0 – indicating pain, but not intolerable pain – the stimulator was selected for testing at that site. The perceived intensity of a single 'pinprick' stimulus was compared with that felt after a series of 10 repetitive 'pinprick' stimuli of the same physical intensity (1/s applied within an area of 1 cm^2). The participant was asked to rate the pain intensity representing the single stimulus and the estimated mean over the whole series of 10 stimuli using a '0–100'

numerical rating scale (NRS). The entire procedure was repeated three times. The WUR was the mean rating of the three series divided by the mean rating of the three single stimuli[8,9].

Pressure Pain Threshold (PPT)

PPT was performed with a digital dynamometer (Kratos, Cotia, Brazil) containing a rod with a 1 cm^2 flat and circular-shaped tip at the end of it, which was used to apply pressure over the masseter in a relaxation posture with an application rate nearly 0.5 kgf cm^{-2} s^{-1}. The participants were instructed to press a button at the first painful sensation. The PPT was determined as the arithmetic mean of three measurements[8,9].

Induction of experimental acute psychological stress

The Paced Auditory Serial Addition Task (PASAT) is known as an effective mental arithmetic task to evoke acute stress[25-27]. Administration of the PASAT involves presenting a series of single digit numbers where the two most recent digits must be summed. For example, if the digits '3', '6' and '2' were presented, the participant would respond with the correct sums, which are '9' and then '8'. The participant must respond prior to the presentation of the next digit for a response to be scored as correct. Four rates of presentation are used (2.4 s, 2.0 s, 1.6 s and 1.2 s) and the task last about 8 min[28] .

Before the task, the participant was informed that the PASAT average performance is about 70–80% correct answers, and that her individual performance should be close or equal to the average performance of all subjects. Finally, the participant was informed that the investigator would be following the performance but could not help or talk during the experiment. The researcher is supposed to give the impression of scoring and taking notes on the subject's performance in order to increase the anxiety. After the end of the session, the investigator informed the participant about a poor performance and asked her to try again and to do her

best. After completion of the second session, the subject received again a negative feedback about her performance.

Procedure

The experimental procedure is described in Figure 1. Each subject was invited to a single testing session that lasted approximately 3 hours. Testing took place in a quiet room and a pleasant temperature was maintained all the time. The individual was seated in a comfortable armchair. After signing an informed consent and answer the psychological assessment, all participant received instructions prior to the QST assessment.

The data were obtained in 3 times. *Baseline (pre stress):* the subject was asked to rest quietly for 10 minutes and the first stress VAS was obtained. After that, the subject underwent a QST battery. *Stress:* the subjects received the preparatory explanations for the PASAT task, after which the software was run for 8 minutes. At the end of the task, the participants received a negative feedback about their performance and were asked to perform the task again for an additional 8 minutes, after which they again received negative feedback about their performance. Immediately upon completion of the two PASAT tasks, the second VAS was obtained in order to evaluate whether the manipulation induced the perceived stress. Afterwards, the same QST battery was performed in the same order. *Post stress:* upon completion of the QST measurements, the subjects received an explanation of the true purpose of the PASAT and were assured that their performance was not negative. They were asked to relax and rest. Approximately 20-30 minutes after the reassurance, the third stress VAS was obtained and the third QST battery was performed.

Statistical analysis

Quantitative variables were reported as means ± standard deviation (SD) and they were assessed for normal distribution using the Kolmogorov-Smirnov test and a log10

transformation was performed when the test results were significant, considering an alpha level of 5% ($p<0.050$). Thus, the following variables were log10 transformed: VAS and psychological assessment scores, absolute values, i.e., raw data, of the CDT, WDT, CPT, HPT, MPT, WUR and PPT.

Repeated measures analysis of variance (ANOVA) was performed to assess the effect of group and time on the reported stress and absolute values of QST. When appropriate, post hoc analyses were performed using Tukey's Honestly Statistical Difference (HSD). The significance level was set at 5% ($p=0.050$).

In addition, QST parameters were transformed into z-values according to the following expression: $Z\text{-score} = (\text{Value}_{\text{single}} - \text{Mean baseline}_{\text{healthy}}) / \text{SD baseline}_{\text{healthy}}$. A z-score of 0 ± 1.96 represents the interval which includes 95% of the baseline data. Positive z-scores denoted a gain of function for the tested stimuli (hyperesthesia, hyperalgesia), whereas negative z-scores denoted a loss of function (hypoesthesia, hypoalgesia). A z-score of 0 corresponds to the mean value of the participants at baseline. Repeated measures analysis of variance (ANOVA) was performed to assess the effect of group and time on the z-scores. When appropriate, post hoc analyses were performed using Tukey's Honestly Statistical Difference (HSD). The significance level was set at 5% ($p=0.05$).

RESULTS

Demographic and Psychological assessment

There was no significant difference in the age between healthy volunteers and TMD patients ($p = 0.183$). Both groups reported similar amount of anxiety ($p = 0.312$ for trait dimension and $p = 0.097$ for state dimension), perceived stress ($p = 0.744$) and pain catastrophization ($p = 0.356$) (Table 1).

Stress assessment

The reported stress showed main effects of time (ANOVA: $F=33.9$, $p<0.001$). Both groups showed an increased stress level following PASAT (Tukey: $p<0.001$), which decreased in post stress condition (Table 2).

Quantitative Sensory Testing

The QST descriptive data are shown in Table 2. CDT and WDT absolute values showed main effects of time (CDT, ANOVA: $F=4.25$, $p=0.017$ and WDT, ANOVA: $F=4.10$, $p=0.020$). After exposure to the stressor, both groups showed significant increase in thermal detection threshold when compared to baseline (CDT, Tukey: $p=0.012$ and WDT, Tukey: $p=0.040$). Furthermore, CPT and HPT showed main effects of time (CPT, ANOVA: $F=11.2$, $p<0.001$ and HPT, ANOVA: $F=8.13$, $p<0.001$). Both groups presented decrease in thermal pain threshold at stress (CPT, Tukey: $p<0.001$ and HPT, Tukey: $p=0.001$) and post stress conditioning (CPT, Tukey: $p=0.005$ and HPT, Tukey: $p=0.006$) when compared to baseline. However, stress did not induce significant changes in MPT, PPT or WUR in both groups ($p>0.050$).

TMD patients showed increased pain sensitivity to mechanical stimuli when compared to healthy volunteers (MPT, ANOVA: $F=4.65$, $p=0.037$), (PPT, ANOVA: $F=6.4$, $p=0.015$ and WUR, ANOVA: $F=33.1$, $p<0.001$). WUR also showed a significant interaction between group and time (ANOVA: $F=17.6$, $p<0.001$) where the TMD group showed higher ratios (Tukey: $p<0.018$) and the healthy volunteers presented lowest ratios (Tukey: $p<0.001$) at baseline.

Similar results were also found when analyzing the z-scores. CDT and WDT showed loss of function for both groups (CDT, ANOVA: $F=4.25$, $p=0.017$ and WDT, ANOVA: $F=4.10$, $p=0.020$) after stress conditioning when compared to baseline (CDT, Tukey: $p=0.012$ and

WDT, Tukey: p=0.040). Significant gain of function was identified for CPT (ANOVA: F=11.2, p<0.001) and HPT (ANOVA: F=8.12, p<0.001) for both groups at stress (CPT, Tukey: p<0.001 and HPT, Tukey: p=0.001) and at post stress conditioning (CPT, Tukey: p=0.005 and HPT, Tukey: p=0.006). The TMD group presented higher z-scores for MPT (ANOVA: F=3.15; Tukey: p=0.037), PPT (ANOVA: F=6.4, p=0.015) and WUR (ANOVA: F=33.1, p<0.001), with a significant difference between group and time (ANOVA: F=17.6, p<0.001) where the TMD group presented the highest WUR z-scores at baseline (Tukey: p<0.018), whereas the healthy volunteers the lowest WUR z-scores at baseline (Tukey: p<0.001). Finally, individual abnormal z-scores were not detected in TMD patients (Figure 2).

DISCUSSION

The main objective of the study was to investigate the effects of experimental psychological stress on QST response in TMD patients and healthy volunteers. Psychological stress was expected to affect QST response and in a different mode between TMD patients and healthy volunteers.

The results of this study revealed that stress affects the thermal thresholds on masticatory muscle, regardless of the presence of TMD. After exposure to an acute mental stressor, both groups developed thermal hypoesthesia (CDT, WDT) and thermal hyperalgesia (CPT, HPT). No changes were detected in mechanical pain sensitivity (MPT, PPT, WUR) after the stress task. These findings of alterations in thermal thresholds and no changes in mechanical pain sensitivity suggest a stress-induced peripheral sensitization rather than central changes on pain pathways[29-31]. In addition, TMD patients were not differently affected by stress in comparison with healthy volunteers, which may be related to the fact that both groups reported similar levels of psychological distress. Psychological factors including stress, depression, anxiety

and pain catastrophizing have also been related to QST responses, with those reporting increased psychological vulnerability generally showing more sensitivity to noxious stimuli and altered detection thresholds[32,33].

Participants reported a significant increase in perceived stress following the PASAT. This is in agreement with previous reports showing that this mental arithmetic task to evoke acute stress[25-27]. Although a physiological response marker to stress was not used in this study, previous studies demonstrated the activation of the sympathetic nervous system[26,27] and hypothalamic-pituitary adrenocortical axis[34,35] by PASAT. In addition, the current stress concept maintains the view that stress should be primarily considered as a cognitive perception of uncontrollability and/or unpredictability that is expressed in a physiological and behavioral response. Moreover, the reverse is not always true; the physiological response by itself does not necessarily indicate a state of stress[36].

QST allows the assessment of specific sensory modalities, which correspond to distinct receptors, peripheral nerve fibers, and their corresponding central nervous system pathways[6,8]. As a measure of Aδ-fiber function, CDT, CPT, HPT and MPT were assessed. C-fiber function was tested by assessing the WDT. PPT was the test for deep pain sensitivity, mediated by muscle C and Aδ-fibers, while WUR represents a psychophysical measure of central sensitization.

The current study found stress-induced thermal hypoesthesia, which as far as we know, has not previously been described in human. Animal studies have reported that thermal hypoesthesia observed following the experimental psychological stress could be attributed to increase of body temperature. Stimulation of the sympathetic axis increases the body temperature by activating β3-adrenoceptor in brown adipose tissue and α-adrenoceptor-mediated peripheral vasoconstriction to inhibit heat loss[37,38]. Several studies have demonstrated that acute psychological stress induces hyperthermia via proinflammatory

cytokine- and PGE2- independent mechanisms[39-41]. In summary, a general warming of the skin is observed due to the increasing of the body temperature, at the same time that the peripheral vasoconstriction induces a marked cooling of extremities (hands, fingers, and feet). Another important finding of this study was stress-induced thermal hyperalgesia in both groups. Studies on humans have shown that experimentally psychological stress can elicits hypoalgesia[42,43] and hyperalgesia[44-46] reactions. The direction of this effect, however, depends on many factors: the nature and persistence of the stressor, sex of the stressed individuals, physiological state of the stress system, the psychological effects that the stressor exerts on the individual's emotions and interactions among these factors[47,48]. Rhudy and colleagues[49-51] have demonstrated that, depending on the amount of arousal (high or low) and the type of emotional response (fear or anxiety), these stressors can lead to hypo or hyperalgesia. Hypoalgesic effects have typically been induced by the stressor that is intense and with high arousal, such as fear. In contrast, when the stressor is less intense and with low to moderate arousal, an anxious state and hyperalgesia are observed. The hypoalgesic effect is thought to facilitate escape from immediate danger while hyperalgesic effects are often discussed in psychological terms, such as stress increasing vigilance to aversive stimuli, as pain[49]. However, the findings of the present study do not support this explanation, because if the anxiety had triggered the thermal hyperalgesia, it should have affected the all other stimuli as MPT, PPT and WUR.

The present results are consistent with the stress effect in peripheral sensitization of cutaneous nociceptors fibers leading to thermal hyperalgesia. A possible explanation for this might be the release of pro-inflammatory cytokines mediated by Sympathetic Nervous system (SNS) activation during exposure to stressors[52]. Activation of the SNS is associated with release of catecholamines from the adrenal medulla that activate the production of IL-1β; IL-6; TNF-α; and IL-10 by immune cells. These pro-inflammatory cytokines are able to exert a sensitization

effect on cutaneous nociceptors fibers, such as Aδ-fiber and C-fiber which mediate the thermal stimuli[53]. Although our study does not provide autonomic variables responses or measurement of catecholamines, a preclinical study demonstrated that the stress-induced thermal hyperalgesia requires SNS activity[54].

Another expected finding was that, regardless of the effect of stress, TMD patients showed an enhanced sensitivity to pinprick (MPT), pain pressure (PPT) stimuli and enhanced temporal summation of pain (WUR) compared to healthy control subjects at the baseline testing. The observation that TMD patients are more sensitive to noxious stimuli, even remote from where the ongoing pain is experienced, have been demonstrated by recent studies using standardized QST protocol[8,9] and support the hypothesis of an alteration of the central pain processing mechanisms resulting in central sensitization[55-57]. In addition, a deficiency in pain inhibitory mechanisms could also explain the reduced pain thresholds in the TMD patients with myofascial pain[58,59] , although not discussed here.

No evidence of stress-induced changes in mechanical pain sensitivity was detected in healthy and TMD subjects. As previously stated, TMD patients included in this investigation presented an alteration of the processing of mechanical stimulus, since they did display an increased sensitivity to mechanical stimulation at the baseline testing. Because stress affects pain processing throughout the central and peripheral mechanisms[52,60], it was expected that stress could aggravate abnormal pain processing in TMD. The results suggest that the central sensitization in TMD is not affect by experimental acute stress. Another explanation is that mechanical pain sensitivity could not be detected probably due to low detecting power of the QST in subjects who are already sensitized and already feel pain under conditions of low intensity mechanical stimulation.

Although individual abnormal z-scores were not detected in TMD patients, somatosensory abnormalities in terms of gain of function for mechanical stimuli were observed in

conventional group comparisons. In order to allow clinical judgments, the DFNS recommends on single case basis comparisons[61]. If the individual *z*-values are outside of the 95% confidence interval of the reference group (± 1.96) the values are considered as abnormal. Given that some patients show increased, while others show decreased responses, group mean comparisons could give false-negative normal values[61]. In the other hand, when conventional group comparisons are made, if the mean of a TMD group is within the Z-scores interval, this does not imply that it does not differ from a healthy group. Values are significantly different from those of healthy subjects, if their 95% confidence interval does not cross the zero line[62], such as MPT, PPT and WUR found in this study.

The present study has some limitations. First, the subjects were tested to QST in the same area on masseter muscle, so it cannot be completely ruled out the increases of pain sensitivity might be related to unlikely but possible local inflammation due to the repetitive stimulation. Second, only women were investigated. It remains unclear whether men respond similarly or not in terms of pain sensitivity during stress situations. Therefore, our results cannot be extrapolated to male population. At last, physiological response of stress was not measured in this study. Although the current view that stress is primarily a perceived phenomenon and a subjective evaluation is essential to ascertain a stress response, autonomic variables and measurement of cortisol levels could give additional information about how the stress system affects the pain sensitivity.

CONCLUSIONS

This study demonstrates that experimental psychological stress induces thermal hypoesthesia and thermal hyperalgesia on masticatory muscle, regardless of the presence of TMD painful. The changes on thermal QST are probability result of the SNS activation following exposure

to stressors. Overall, these findings emphasize the importance of considering the psychological stress when judging QST findings. Although we have used a visual analogic scale to assessment the perceived stress, a construct with some state-like characteristics could be more appropriate. Additional studies should be carried out to replicate these findings and to investigate the effect of emotional states on QST measurements.

REFERENCES

1. de Leeuw R, Klasser GD. Diagnosis and Management of TMDs. In: Orofacial Pain: Guidelines for Assessment, Diagnosis, and Management. Fifth Edition. Chicago: Quintessence, 2013: 127-185.

2. National Institute of Dental and Craniofacial Research. Facial Pain. http://www.nidcr.nih.gov/DataStatistics/FindDataByTopic/FacialPain/. Acess in May 2017.

3. Durham J, Shen J, Breckons M, Steele JG, Araujo-Soares V, Exley C, Vale L. Healthcare Cost and Impact of Persistent Orofacial Pain: The DEEP Study Cohort. J Dent Res 2016; 95:1147-54.

4. Lobbezoo F, Drangsholt M, Peck C, Sato H, Kopp S, Svensson P. Topical review: new insights into the pathology and diagnosis of disorders of the temporomandibular joint. J Orofac Pain 2004;18:181–91.

5. Woolf CJ. Central sensitization: implications for the diagnosis and treatment of pain. Pain 2011;152:S2–15.

6. Backonja MM, Attal N, Baron R, Bouhassira D, Drangholt M, Dyck PJ, et al. Value of quantitative sensory testing in neurological and pain disorders: NeuPSIG consensus. Pain 2013;154:1807-19.

7. Pavlaković G, Petzke F. The role of quantitative sensory testing in the evaluation of musculoskeletal pain conditions. Curr Rheumatol Rep 2010;12:455-61.

8. Rolke R, Magerl W, Campbell KA, Schalber C, Caspari S, Birklein F, et al Quantitative sensory testing: a comprehensive protocol for clinical trials. Eur J Pain 2006;10:77.

9. Rolke R, Baron R, Maier C, Tölle TR, Treede RD, Beyer A, et al. Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): standardized protocol and reference values. Pain 2006;123:231–43.

10. Kothari SF, Baad-Hansen L, Oono Y, Svensson P. Somatosensory assessment and conditioned pain modulation in temporomandibular disorders pain patients. Pain. 2015;156:2545-55.

11. Yang G, Baad-Hansen L, Wang K, Fu K, Xie QF, Svensson P. Somatosensory abnormalities in Chinese patients with painful temporomandibular disorders. J Headache Pain 2016;17:31.

12. Pfau DB, Rolke R, Nickel R, Treede RD, Daublaender M et al. Somatosensory profiles in subgroups of patients with myogenic temporomandibular disorders and Fibromyalgia Syndrome. Pain 2009; 15;147(1-3):72-83.

13. Backonja MM, Walk D, Edwards RR, Sehgal N, Moeller-Bertram T, Wasan A, Irving G, Argoff C, Wallace M. Quantitative sensory testing in measurement of neuropathic pain phenomena and other sensory abnormalities. Clin J Pain 2009;25(7):641-7.

14. Pigg M, Baad-Hansen L, Svensson P, Drangsholt M, List T. Reliability of intraoral quantitative sensory testing (QST). Pain 2010;148:220–226.

15. Costa YM, Morita-Neto O, de Araújo-Júnior EN, Sampaio FA, Conti PC, Bonjardim LR. Test-retest reliability of quantitative sensory testing for mechanical somatosensory and pain modulation assessment of masticatory structures. J Oral Rehabil 2017; 44:197-204.

16. Marcuzzi A, Wrigley PJ, Dean CM, Adams R, Hush JM. The long-term reliability of static and dynamic quantitative sensory testing in healthy individuals. Pain 2017;158:1217-1223.

17. Wolf OT. Stress and memory in humans: twelve years of progress? Brain Res. 2009;1293:142-54.

18. Shields GS, Sazma MA, McCullough AM, Yonelinas AP. The effects of acute stress on episodic memory: A meta-analysis and integrative review. Psychol Bull 2017;143:636-675.

19. Racine M, Tousignant-Laflamme Y, Kloda LA, Dion D, Dupuis G, Choinière M. A systematic literature review of 10 years of research on sex/gender and experimental pain perception – part 1: are there really differences between women and men? Pain 2012;153:602-18.

20. Dworkin SF, LeResche L. Research diagnostic criteria for temporomandibular disorders: review, criteria, examinations and specifications, critique. J Craniomandib Disord 1992;6: 301–355.

21. Luft CD, Sanches S de O, Mazo GZ, Andrade A. Brazilian version of the Perceived Stress Scale: translation and validation for the elderly. Rev Saude Publica 2007; 41:606-615.

22. Gorenstein C, Andrade L. Validation of a Portuguese version of the Beck Depression Inventory and the State-Trait Anxiety Inventory in Brazilian subjects. Braz J Med Biol Res 1996;29:453-7.

23. Sehn F, Chachamovich E, Vidor LP, Dall-Agnol L, de Souza IC, Torres IL, et al. Cross-cultural adaptation and validation of the Brazilian Portuguese version of the pain catastrophizing scale. Pain Med 2012;13:1425-35.

24. Costa YM, Morita-Neto O, de Araújo-Júnior EN[1], Sampaio FA, Conti PC, Bonjardim LR. Test-retest reliability of quantitative sensory testing for mechanical somatosensory and pain modulation assessment of masticatory structures. J Oral Rehabil 2017;44:197-204.

25. Tombaugh TN. A comprehensive review of the Paced Auditory Serial Addition Test (PASAT). Arch Clin Neuropsychol 2006;21:53–76.

26. Tanosoto T, Arima T, Tomonaga A, Ohata N, Svensson P. A Paced Auditory Serial Addition Task evokes stress and differential effects on masseter-muscle activity and haemodynamics. Eur J Oral Sci 2012;120:363-7.

27. Tanosoto T, Bendixen KH, Arima T, Hansen J, Terkelsen AJ, Svensson P. Effects of the Paced Auditory Serial Addition Task (PASAT) with different rates on autonomic nervous system responses and self-reported levels of stress. J Oral Rehabil 2015;42:378-85.

28. Gronwall, DMA. Paced auditory serial-addition task: a measure of recovery from concussion. Percep Mot Skills 1977;44: 367-73.

29. Treede RD, Meyer RA, Raja SN, Campbell JN. Peripheral and central mechanisms of cutaneous hyperalgesia. Prog Neurobiol 1992;38: 397–421.

30. LaMotte RH, Thalhammer JG, Torebjork HE, Robinson CJ. Peripheral neural mechanisms of cutaneous hyperalgesia following mild injury by heat. J Neurosci 1982;2:765–81.

31. Simone DA, Sorkin LS, Oh U, Chung JM, Owens C, LaMotte RH, et al. Neurogenic hyperalgesia: central neural correlates in responses of spinothalamic tract neurons. J Neurophysiol 1991;66:228–46.

32. Wallin M, Liedberg G, Börsbo B, Gerdle B.Thermal detection and pain thresholds but not pressure pain thresholds are correlated with psychological factors in women with chronic whiplash-associated pain. Clin J Pain. 2012; 28:211-21.

33. Hübscher M, Moloney N, Rebbeck T, Traeger A, Refshauge KM. Contributions of mood, pain catastrophizing, and cold hyperalgesia in acute and chronic low back pain: a comparison with pain-free controls. Clin J Pain 2014;30:886-93.

34. Girdler SS, Pedersen CA, Straneva PA, Leserman J, Stanwyck CL, Benjamin S, et al. Dysregulation of cardiovascular and neuroendocrine responses to stress in premenstrual dysphoric disorder. Psychiatry Res 1998; 81:163-78.

35. Lustyk MK, Olson KC, Gerrish WG, Holder A, Widman L. Psychophysiological and neuroendocrine responses to laboratory stressors in women: implications of menstrual cycle phase and stressor type. Biol Psychol 2010; 83:84-92.

36. Koolhaas JM, Bartolomucci A, Buwalda B, de Boer SF, Flügge G, Korte SM, et al. Stress revisited: a critical evaluation of the stress concept. Neurosci Biobehav 2011;35:1291-301.

37. Gordon CJ. Thermal biology of the laboratory rat. Physiol Beh 1990; 47: 963–991.

38. Vianna DM, Carrive P. Changes in cutaneous and body temperature during and after conditioned fear to context in the rat. Eur J Neurosci 2005;21:2505-12.

39. Oka T, Oka K, Kobayashi T, Sugimoto Y, Ichikawa A, Ushikubi F, Narumiya S, Saper CB. Characteristics of thermoregulatory and febrile responses in mice deficient in prostaglandin EP1 and EP3 receptors. J Physiol 2003; 551:945-54;

40. Lkhagvasuren B, Nakamura Y, Oka T, Sudo N, Nakamura K. Social defeat stress induces hyperthermia through activation of thermoregulatory sympathetic premotor neurons in the medullary raphe region. Eur J Neurosci 2011; 34:1442-52.

41. Lkhagvasuren B, Oka T, Nakamura Y, Hayashi H, Sudo N, Nakamura K. Distribution of Fos-immunoreactive cells in rat forebrain and midbrain following social defeat stress and diazepam treatment. Neuroscience 2014; 272:34-57.

42. Diener SJ, Wessa M, Ridder S, Lang S, Diers M, Steil R, et al. Enhanced stress analgesia to a cognitively demanding task in patients with posttraumatic stress disorder. J Affect Dis 2012;136:1247–51.

43. Yilmaz P, Diers M, Diener S, Rance M, Wessa M, Flor H. Brain correlates of stress-induced analgesia. Pain 2010;151:522–9.

44. Vedolin GM, Lobato VV, Conti PC, Lauris JR. The impact of stress and anxiety on the pressure pain threshold of myofascial pain patients. J Oral Rehabil 2010;36:313-21.

45. Crettaz B, Marziniak M, Willeke P, Young P, Hellhammer D, Stumpf A, et al. Stress-induced allodynia - evidence of increased pain sensitivity in healthy humans and patients with chronic pain after experimentally induced psychosocial stress. Plos One 2013; 8:e69460.

46. Reinhardt T, Kleindienst N, Treede RD, Bohus M, Schmahl C. Individual modulation of pain sensitivity under stress. Pain Med 2013;14:676-85.

47. Chrousos GP. Stress and disorders of the stress system. Nat Rev Endocrinol 2009;5:374–381.

48. Levine FM, Krass SM, Padawar WJ. Failure hurts: the effects of stress due to difficult tasks and failure feedback on pain report. Pain 1993;54:335–40.

49. Rhudy JL, Meagher MW. Fear and anxiety: divergent effects on human pain thresholds. Pain 2000;84:65–75.

50. Rhudy JL, Meagher MW. Negative affect: effects on an evaluative measure of human pain. Pain 200;104 : 617-626.

51. Rhudy JL, Williams AE, McCabe KM, Russell JL, Maynard LJ. Emotional control of nociceptive reactions (ECON): do affective valence and arousal play a role? Pain 2008; 136: 250-261

52. Jennings EM, Okine BN, Roche M, Finn DP. Stress-induced hyperalgesia. Prog Neurobiol 2014;121:1-18.

53. Marsland AL, Walsh C, Lockwood K, John-Henderson NA. The effects of acute psychological stress on circulating and stimulated inflammatory markers: A systematic review and meta-analysis. Brain Behav Immun 2017;12.

54. Donello JE, Guan Y, Tian M, Cheevers CV, Alcantara M, Cabrera S,et al. A peripheral adrenoceptor-mediated sympathetic mechanism can transform stress-induced analgesia into hyperalgesia. Anesthesiology 2011;114:1403-16.

55. Giesecke T, Gracely RH, Grant MA, et al. Evidence of augmented central pain processing in idiopathic chronic low back pain. Arthritis Rheum 2004;50:613–623.

56. Herrero JF, Laird JM, Lopez-Garcia JA. Wind-up of spinal cord neurones and pain sensation: much ado about something? Prog Neurobiol 2000;61:169–203.

57. Woolf CJ. Central sensitization: implications for the diagnosis and treatment of pain. Pain 2011;152:S2–S15.

58. King CD, Wong F, Currie T, Mauderli AP, Fillingim RB, Riley JL. Deficiency in endogenous modulation of prolonged heat pain in patients with Irritable Bowel Syndrome and Temporomandibular Disorder. Pain 2009;143:172-8.

59. Hilgenberg-Sydney PB, Kowacs PA, Conti PC. Somatosensory evaluation in Dysfunctional Syndrome patients. J Oral Rehabil 2016;43:89-95

60. Johnson AC, Greenwood-Van Meerveld B. Stress-induced pain: a target for the development of novel therapeutics. J Pharmacol Exp Ther. 2014; 51:327-35.

61. Maier C, Baron R, Tölle TR, Binder A, Birbaumer N, Birklein F, et al. Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): somatosensory abnormalities in 1236 patients with different neuropathic pain syndromes. Pain 2010;150:439–50.

62. Baron R, Maier C, Attal N, et al. Peripheral neuropathic pain: a mechanism-related organizing principle based on sensory profiles. Pain 2017;158:261-272.

FIGURE LEGENDS

Figure 1 – Procedure. Stress VAS = Subject´s rating of stress, QST = Quantitative Sensory Testing, PASAT = Paced Auditory Serial Addition Task

Figure 2 – The Z-score profiles of 20 TMD patients and 20 healthy volunteers for all tested QST parameters at masseter area before and after stress test. A= thermal detection profiles. B= thermal pain profiles. C= mechanical pain profiles. D= temporal summation profiles

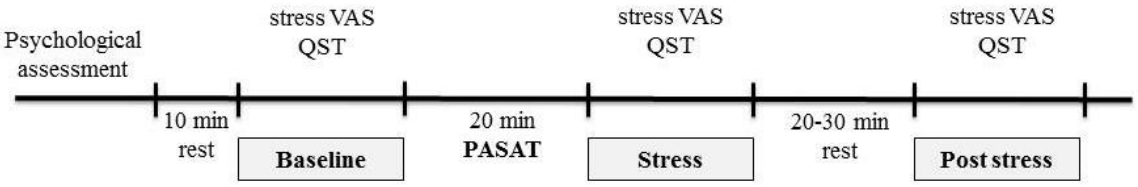
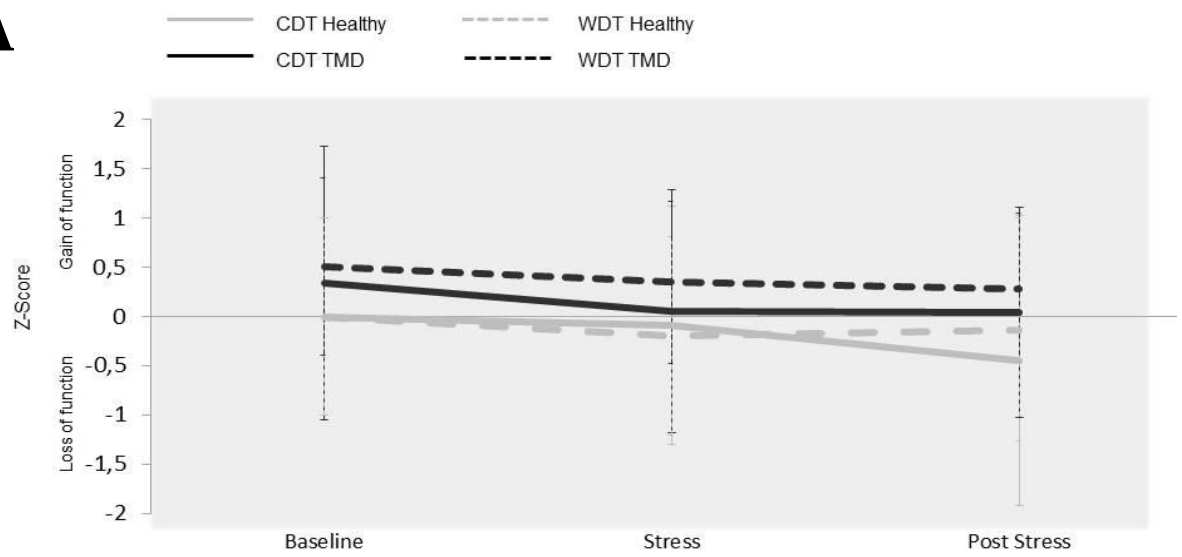

Psychological assessment
stress VAS
QST
stress VAS
QST
stress VAS
QST
10 min rest
Baseline
20 min
PASAT
Stress
20-30 min rest
Post stress

Fig 1

A


CDT Healthy
CDT TMD
WDT Healthy
WDT TMD
Z-Score
Gain of function
Loss of function
2
1,5
1
0,5
0
-0,5
-1
-1,5
-2
Baseline
Stress
Post Stress

B

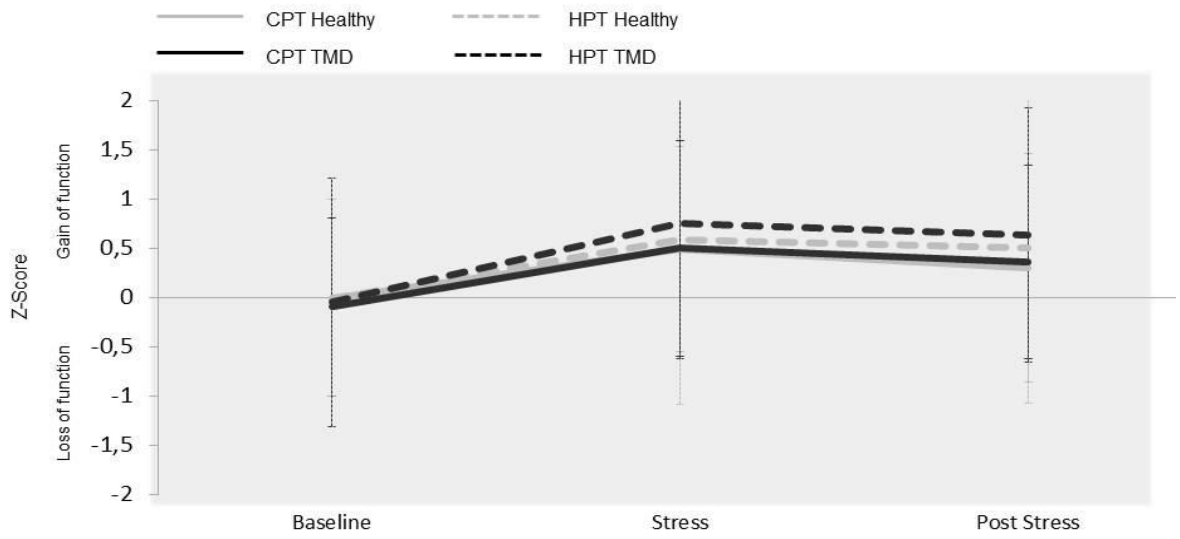
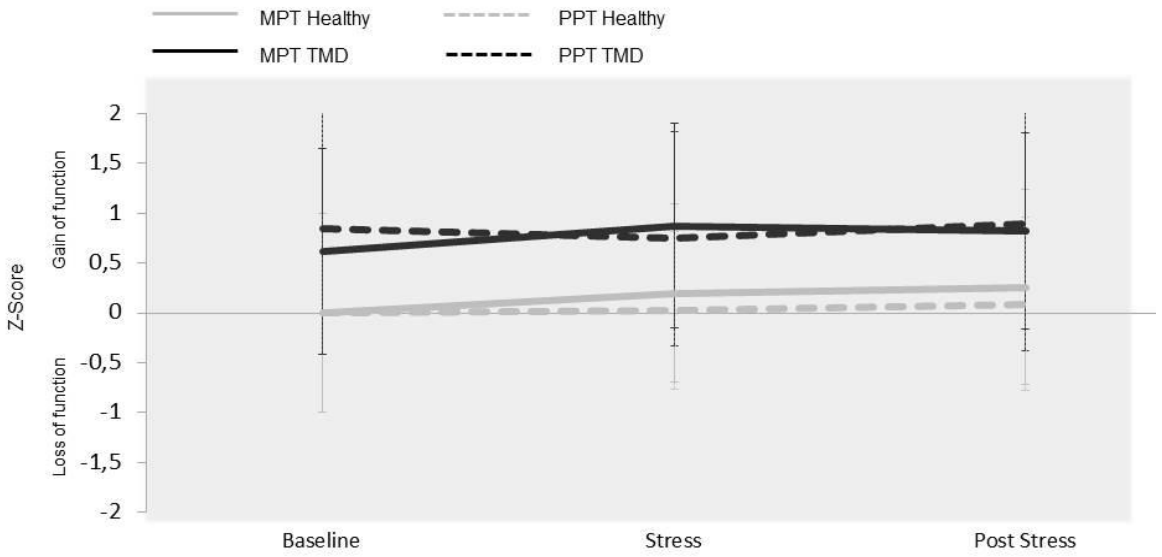

CPT Healthy
CPT TMD
HPT Healthy
HPT TMD
Z-Score
Gain of function
Loss of function
2
1,5
1
0,5
0
-0,5
-1
-1,5
-2
Baseline
Stress
Post Stress

Fig 2

C



D

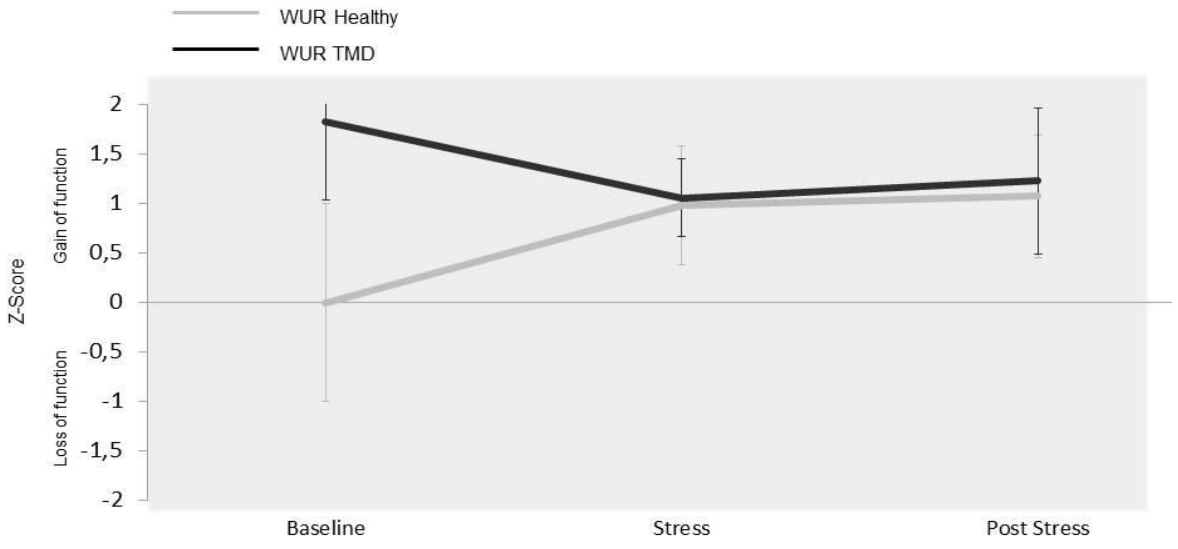


Fig 2 (continuation)

Table 1. Demographical data and psychological assessment.

	Healthy Volunteers **mean (SD)**	**TMD** **mean (SD)**	**P-value**
Age (yrs)	29.45 (6.67)	30.10 (9.11)	0.183
STAI trait dimension (0-80)	43.80 (3.81)	40.65 (6.36)	0.312
STAI state dimension (0-80)	43.70 (4.70)	40.90 (6.94)	0.097
PSS (0-56)	23.50 (8.23)	21.65 (7.18)	0.744
PCS (0-52)	12.30 (9.97)	22.85 (14.18)	0.356

STAI = State-Trait Anxiety Inventory, PSS = Perceived Stress Scale, PCS = Pain Catastrophizing Scale.

Table 2. Reported Stress level and Quantitative Sensory Testing (QST) obtained in the 3 times of the study.

	Baseline mean (SD)	**Stress** mean (SD)	**Post stress** mean (SD)
Healthy Volunteers			
Reported Stress			
stress VAS	1.61 (1.97)	5.16 (1.89)	1.68 (1.53)
QST			
CDT (°C)	27.60 (2.84)	27.39 (3.32)	26.45 (3.70)
WDT (°C)	38,87 (3.58)	39.58 (3.72)	39.40 (4.18)
CPT (°C)	10.58 (8.49)	14.78 (8.84)	13.15 (9.87)
HPT (°C)	45.98 (2.08)	44.74 (3.51)	44.92 (3.30)
MPT (g/mm^{-2})	47.67 (63.29)	26.87 (29.48)	27.56 (32.67)
WUR (NRS)	1.08 (1.11)	1.73 (0.71)	1.85 (0.80)
PPT (kgf/cm^2)	1.13 (0.35)	1.10 (0.27)	1.08 (0.25)
TMD Patients			
Reported Stress			
stress VAS	1.24 (1.60)	2.77 (1.83)	0.97 (1.20)
QST			
CDT (°C)	28.72 (3.35)	27.83 (3.30)	27.73 (2.80)
WDT (°C)	37,07 (3.22)	37.59 (2.94)	37.79 (2.69)
CPT (°C)	9.80 (7.69)	14.84 (9.28)	13.70 (8.34)
HPT (°C)	46.08 (2.63)	44.40 (2.87)	44.65 (2.69)
MPT (g/mm^{-2})	25.52 (53.81)	22.98 (57.82)	16.96 (40.41)
WUR (NRS)	3.12 (1.40)	1.74 (0.44)	2.13 (1.22)
PPT (kgf/cm^{-2})	0.90 (0.29)	0.91 (0.26)	0.89 (0.32)

VAS= visual analog scale, CDT= cold detection threshold, WDT= warm detection threshold, CPT= cold pain threshold, HDT= heat pain threshold, MPT= mechanical pain threshold, WUR= wind-up ratio, PPT= pressure pain threshold

3 Discussion

3 DISCUSSION

The present study was designed to investigate the effects of experimental psychological stress on QST response in TMD patients and healthy individuals using a standardized QST protocol. Based in the most frequent somatosensory abnormalities in TMD patients, we focused on 4 thermal and 3 mechanical psychophysical tests. The experimental hypothesis to be tested was that psychological stress would affect the QST response and that such would be TMD patients would be differently affected when compared to healthy volunteers.

In line with our hypotheses, the results of this study revealed that stress affects the thermal thresholds on masticatory muscle. After exposure to an acute mental stressor, both groups developed thermal hypoesthesia (CDT, WDT) and thermal hyperalgesia (CPT, HPT). There were no changes of mechanical pain sensitivity (MPT, PPT, WUR) after the stress task. This finding of stress affects the thermal thresholds and no changes in mechanical pain sensitivity suggest a stress-induced peripheral sensitization rather than central changes on pain pathways[41-44].

Furthermore, TMD patients were not differently affected by stress in comparison with healthy volunteers, which may be related to the fact that both groups reported similar levels of psychological distress. Psychological factors including stress, depression, anxiety and pain catastrophizing has also been related to QST responses, with those reporting increased psychological vulnerability generally showing more sensitivity to noxious stimuli and altered detection thresholds[45,46].

Experimental stress test

Our participants reported a significant increase in perceived stress following the PASAT. This is in agreement with previous reports showing that this mental arithmetic task to evoke acute stress[47-49]. Although a physiological response marker to stress was not adopted in this study, previous researches demonstrate the activation of the sympathetic nervous system[48-50] and hypothalamic-pituitary adrenocortical axis[51-53] by PASAT. In addition, the

current stress concept maintains the view that stress should be primarily considered as a cognitive perception of uncontrollability and/or unpredictability that is expressed in a physiological and behavioral response. Moreover, the reverse is not always true; the physiological response by itself does not necessarily always indicate a state of stress[35].

Group differences in pain sensitivity

QST allows the assessment of specific sensory modalities, which correspond to distinct receptors, peripheral nerve fibers, and their corresponding central nervous system pathways[11,12]. It assesses sensory aspects of cutaneous as well as deep pain (muscles, fascia and ligaments) [13]. As a measure of Aδ-fiber function, CDT, CPT, HPT and MPT were assessed. C-fiber function was tested by assessing the WDT. PPT was the test for deep pain sensitivity, mediated by muscle C and Aδ fibers and WUR represents a psychophysical measure of central sensitization.

An important finding was that TMD patients showed an enhanced sensitivity to pinprick (MPT), pressure (PPT) stimuli and higher ratio of temporal summation of pain (WUR) compared to healthy control subjects at the baseline testing. Recent studies using standardized QST protocol have found that, compared with pain-free controls, TMD patients show evidence of pain hypersensitivity with decreased pain threshold at the primary area of pain as well as at sites remote from the primary site of pain[18-20]. Increased pain sensitivity in the primary area of pain (local pain) is considered a sign of predominantly peripheral pain sensitization, whereas pain sensitivity in areas anatomically remote from the primary area of pain is thought to reflect a more central phenomenon[21-23]. Furthermore, the temporal summation, that is responses to repeated noxious stimuli, is considered a reflection of "wind up" and represents a psychophysical measure of central sensitization[23-25]. In addition, a deficiency in pain inhibitory mechanisms could also explain the reduced pain thresholds in the TMD patients with myofascial pain[26,27].

Although individual abnormal z-scores were not detected in TMD patients, somatosensory abnormalities in terms of gain of function for mechanical stimuli were observed in conventional group comparisons. The QST absolutes values are Z-scores transformed to produce a QST profile, where all parameters Z-scores above ''0'' indicate a gain of function when the patient is more sensitive to the tested stimuli compared with

controls (hyperesthesia, hyperalgesia), while Z-scores below ‘‘0’’ indicate a loss of function referring to a lower sensitivity of the patient (hypoesthesia, hypoalgesia)[12]. Therefore, the z-score profiles give a quick overview of any somatosensory loss or gain in QST stimuli modalities. A Z-score of zero indicated an individual value corresponding to the group mean of the healthy control subjects. The Z-scores of ± 1.96 represents the range that would be expected to include 95% of the healthy control data and, to single-case comparisons, any Z-scores outside this interval are considered as abnormal[12]. In order to allow clinical judgments, the DFNS recommends on single-case comparisons with the control group data instead to conventional group comparisons[54]. Given that some TMD patients show increased, while others show decreased responses, group mean comparisons could give false-negative normal values. In the other hand, when conventional group comparisons are made, if the mean of a TMD group is within the Z-scores of ± 1.96 interval, this does not imply that it does not differ from a healthy group. Values are significantly different from those of healthy subjects, if their 95% confidence interval does not cross the zero line[55], such as MPT, PPT and WUR found in this study.

Effect of stress on QST response

The current study found stress-induced thermal hypoesthesia (increase in thermal detection thresholds, CDT and WDT), however, as far as we know, this result has not previously been described in human, but could be attributed to increase of body temperature. Animal studies have demonstrated that many types of acute psychological stress increase body temperature, such as being placed into an unfamiliar space or an open field, changing home cages, restraint/immobilization and exposure to dominant animals[56]. The hyperthermia may be attributed to distinct mechanisms. Stimulation of the sympathetic axis increases the body temperature by activating β3-adrenoceptor in brown adipose tissue and α-adrenoceptor-mediated peripheral vasoconstriction to inhibit heat loss[57-59]. Furthermore, several studies have demonstrated that acute psychological stress induces hyperthermia via proinflammatory cytokine- and PGE2- independent mechanisms[60-62]. In summary, a general warming of the skin is observed due to the increasing of the body temperature, at the same time that the peripheral vasoconstriction induces a marked cooling of extremities (hands, fingers, and feet).

Another important finding of this study was stress-induced thermal hyperalgesia (decrease in thermal pain thresholds, CPT and HPT) in both groups. Studies on humans have shown that experimentally psychological stress can elicits hypoalgesia[63,64] and hyperalgesia[65-67] reactions. Whether an individual subjected to stress will exhibit analgesia or hyperalgesia is relatively subjective and seem to be influenced by on the nature and persistence of the stressor, sex of the stressed individuals, physiological state of the stress system, the psychological effects that the stressor exerts on the individual's emotions and interactions among these factors[34,68]. Such factors have led to mixed findings with regard to the effects of stress on pain sensitivity. In humans, physical stimuli such as exercises, cold water immersion, painful stimulation[69-71] as well as psychological stressors such as mental arithmetic[72,73] or public speaking tasks[66,74] or fear[75] have been employed. Rhudy and colleagues have demonstrated that, depending on the amount of arousal (high or low) and the type of emotional response (fear or anxiety), these stressors can lead to hypo- or hyperalgesia[75-77]. Hypoalgesic effects have typically been induced by the stressor that is intense and with high arousal, such as fear. In contrast, when the stressor is less intense and with low to moderate arousal, an anxiety state and hyperalgesia are observed. The hypoalgesic effect is thought to facilitate escape from immediate danger and the hyperalgesic effects are often discussed such stress increasing attention and vigilance to events, including pain. However, the findings of the current study do not support the discussion above. If the anxiety had triggered the thermal hyperalgesia, it should have affected the all other stimuli (MPT, PPT, WUR). Therefore, an anxiety-related effect on thermal hyperalgesia can be ruled out.

The present results are consistent with the stress effect in peripheral sensitization of cutaneous nociceptors fibers leading to thermal hyperalgesia. A possible explanation for this might be the release of pro-inflammatory cytokines mediated by Sympathetic Nervous system (SNS) activation during exposure to stressors[78]. Activation of the SNS is associated with release of catecholamines from the adrenal medulla that activate the production of IL-1β; IL-6; TNF-α; and IL-10 by immune cells. These pro-inflammatory cytokines are able to exert a sensitization effect on cutaneous nociceptors fibers, such as Aδ-fiber and C-fiber which mediate the thermal stimuli[79]. Although our study not provides autonomic variables responses or measurement of catecholamines, a preclinical study demonstrated that the stress-induced thermal hyperalgesia requires the SNS activity[80].

No evidence of stress-induced changes in mechanical pain sensitivity (MPT, PPT, WUR) was detected in healthy and TMD subjects. The enhanced pain to mechanical stimuli is prominent clue of central sensitization and can result from increase input to nervous system or

deficiencies of pain modulation[58]. As previously stated, our TMD patients presented an alteration of the processing of mechanical stimulus, since they did display an increased sensitivity to mechanical stimulation at the baseline testing. Because stress affects pain processing throughout the central and peripheral mechanisms[78,81], one hypothesis is that stress could aggravate abnormal pain processing in TMD. However no effect of experimental stress on mechanical pain sensitivity could be observed in the patients. The results suggest that the central sensitization in TMD is not affect by experimental stress. Another explanation is that the induced stress affects the mechanical pain sensitivity but could not be detected probably due to low detecting power of the QST in subjects who are already sensitized and already feel pain under conditions of low intensity mechanical stimulation.

Limitations

The present experimental study has some limitations. First, the participants were tested to QST in the same area on masseter muscle, so it cannot be completely ruled out the increases of pain sensitivity might be related to unlikely but possible local inflammation due to the repetitive stimulation. Second, only women were investigated in the present study. It remains unclear whether men respond similarly or not in terms of pain sensitivity during stress situations. Therefore, our results cannot be extrapolated to male population. At last, physiological response of stress was not measured in this study. Although the current view that stress is primarily a perceived phenomenon and a subjective evaluation is essential to ascertain a stress response, autonomic variables and measurement of cortisol levels could give additional information about how the stress system affects the pain sensitivity.

Clinical Implications

The pain mechanisms underlying TMD are not fully understood, which complicates the diagnosis and treatment[82,83]. QST has been proposed as a promising tool in the field of pain. It´s ultimate goal is to measure pain and understand its involved processing mechanisms, therefore could be a potential guide for treatment[9,11]. For example, QST TMD

patients with more localized (trigeminal area) somatosensory abnormalities, as localized pain complaint, suggest peripheral pain mechanism that can be treated successfully by local interventions, such as self-management programs, occlusal splints and physiotherapy. In contrast, TMD patients with somatosensory abnormalities outside the trigeminal area and generally increased mechanical pain sensitivity and/or enhanced temporal summation probably present central sensitization, therefore central drugs and an interdisciplinary therapy, besides to local interventions, should be considered. QST measures could be also adopted to predict responses to pain drugs, which is a reality in neuropathic pain field. In a hypothetical case, baseline heat pain threshold could predicts response to gabapentin treatment, but not responses to amitriptyline, in patients with myofascial TMD or TMD patients with impaired conditioned pain modulation (CPM), a dynamic QST, could benefit from agents that augment descending inhibition of pain by spinal monoamine reuptake inhibition such as tricyclic antidepressants.

QST is a complementary method for the clinical assessment and management of the TMD and in combination with assessment of the function of endogenous pain inhibitory pathways by CPM, neuroimaging and genetic may dramatically expanded our understanding of the pain mechanisms underlying the TMD painful and improve the treatment.

Although growing developments of human laboratory research suggests that QST is useful in assessment sensory abnormalities of TMD patients, the QST has not been used as a routine clinical. For QST to be widely accepted and implemented in routine clinical practice there are many areas that still need to be better developed[9,11]. First, the refinements of the standards proposed for conducting QST and development of additional protocols for specific pain conditions, such as musculoskeletal. Second, determination about the influence of cognitive and psychologic factors to individual patients that may affect participation in QST, as addressed by our study. Third, the time demand for testing in routine practice and the expense of the equipment required for some QST. Finally, the lack of information about its clinical utility for musculoskeletal pain. With these additional developments, QST could become a cost effective and clinically useful to TMD pain assessment, which can further the progress toward the goal of mechanism-based personalized pain management.

4 Final Considerations

4 FINAL CONSIDERATIONS

This study demonstrates that experimental psychological stress induces thermal hypoesthesia and thermal hyperalgesia on masticatory muscle, regardless of the presence of TMD painful. The changes on thermal QST are probability result of the SNS activation following exposure to stressors. Overall, these findings emphasize the importance of considering the psychological stress when judging QST findings. Although we have used a Visual Analogic Scale to assess the perceived stress, a construct with some state-like characteristics could be more appropriate. Additional studies should be carried out to replicate these findings and to investigate the effect of emotional states on QST measurements.

References

REFERENCES

1. de Leeuw R, Klasser GD. Diagnosis and Management of TMDs. In: Orofacial Pain: Guidelines for Assessment, Diagnosis, and Management. Fifth Edition. Chicago: Quintessence, 2013: 127-185.

2. National Institute of Dental and Craniofacial Research. Facial Pain. http://www.nidcr.nih.gov/DataStatistics/FindDataByTopic/FacialPain/. Acess in May 2017.

3. Durham J, Shen J, Breckons M, Steele JG, Araujo-Soares V, Exley C, et al. Healthcare Cost and Impact of Persistent Orofacial Pain: The DEEP Study Cohort. J Dent Res 2016; 95:1147-54.

4. Walk D, Poliak-Tunis M. Chronic Pain Management: An Overview of Taxonomy, Conditions Commonly Encountered, and Assessment. Med Clin North Am 2016;100:1-16.

5. Ross E. Moving towards rational pharmacological management of pain with an improved classification system of pain. Expert Opin Pharmacother 2001; 2:1529–1530.

6. Baron R. Mechanisms of disease: neuropathic pain – a clinical perspective. Nat Clin Pract Neurol 2006; 2:95–106.

7. Finnerup NB, Jensen TS. Mechanisms of disease: mechanism-based classification of neuropathic pain-a critical analysis. Nat Clin Pract Neurol 2006; 2:107–115.

8. Vardeh D, Mannion RJ, Woolf CJ. Toward a Mechanism-Based Approach to Pain Diagnosis. J Pain 2016;17:T50-69.

9. Cruz-Almeida Y, Fillingim RB. Can quantitative sensory testing move us closer to mechanism-based pain management? Pain Med 2014; 15:61-72.

10. Yarnitsky D, Granot M. Quantitative sensory testing. Handb Clin Neurol 2006;81:397–409.

11. Backonja MM, Attal N, Baron R, Bouhassira D, Drangholt M, Dyck PJ, et al. Value of quantitative sensory testing in neurological and pain disorders: NeuPSIG consensus. Pain 2013; 154:1807-19.

12. Rolke R, Magerl W, Campbell KA, Schalber C, Caspari S, Birklein F, et al Quantitative sensory testing: a comprehensive protocol for clinical trials. Eur J Pain 2006;10:77.

13. Arendt-Nielsen L, Yarnitsky D. Experimental and clinical applications of quantitative sensory testing applied to skin, muscles and viscera. J Pain 2009; 10:556–72.

14. Basbaum AI, Woolf CJ. Pain. Curr Biol. 1999;9:R429–R431.

15. Backonja MM, Walk D, Edwards RR, Sehgal N, Moeller-Bertram T, Wasan A, et al. Quantitative sensory testing in measurement of neuropathic pain phenomena and other sensory abnormalities. Clin J Pain. 2009;25:641-7.

16. Rolke R, Baron R, Maier C, Tölle TR, Treede RD, Beyer A, et al. Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): standardized protocol and reference values. Pain 2006;123:231–43.

17. Pavlaković G, Petzke F. The role of quantitative sensory testing in the evaluation of musculoskeletal pain conditions. Curr Rheumatol Rep 2010;12:455-61.

18. Kothari SF, Baad-Hansen L, Oono Y, Svensson P. Somatosensory assessment and conditioned pain modulation in temporomandibular disorders pain patients. Pain 2015;156:2545-55.

19. Yang G, Baad-Hansen L, Wang K, Fu K, Xie QF, Svensson P. Somatosensory abnormalities in Chinese patients with painful temporomandibular disorders. J Headache Pain 2016;17:31.

20. Pfau DB, Rolke R, Nickel R, Treede RD, Daublaender M et al. Somatosensory profiles in subgroups of patients with myogenic temporomandibular disorders and Fibromyalgia Syndrome. Pain 2009 15;147:72-83.

21. Giesecke T, Gracely RH, Grant MA, et al. Evidence of augmented central pain processing in idiopathic chronic low back pain. Arthritis Rheum 2004;50:613–623.

22. Herrero JF, Laird JM, Lopez-Garcia JA. Wind-up of spinal cord neurones and pain sensation: much ado about something? Prog Neurobiol 2000;61:169–203.

23. Woolf CJ. Central sensitization: implications for the diagnosis and treatment of pain. Pain 2011;152:S2–S15.

24. Staud R, Robinson ME, Price DD. Temporal summation of second pain and its maintenance are useful for characterizing widespread central sensitization of fibromyalgia patients. J Pain 2007; 8:893–901.

25. Staud R, Price DD, Robinson ME, Mauderli AP, Vierck CJ. Maintenance of windup of second pain requires less frequent stimulation in fibromyalgia patients compared to normal controls. Pain 2004;110:689–696.

26. King CD, Wong F, Currie T, Mauderli AP, Fillingim RB, Riley JL. Deficiency in endogenous modulation of prolonged heat pain in patients with Irritable Bowel Syndrome and Temporomandibular Disorder. Pain 2009;143:172-8.

27. Hilgenberg-Sydney PB, Kowacs PA, Conti PC. Somatosensory evaluation in Dysfunctional Syndrome patients. J Oral Rehabil 2016;43:89-95.

28. Pigg M, Baad-Hansen L, Svensson P, Drangsholt M, List T. Reliability of intraoral quantitative sensory testing (QST). Pain 2010;148:220–226

29. Costa YM, Morita-Neto O, de Araújo-Júnior EN, Sampaio FA, Conti PC, Bonjardim LR. Test-retest reliability of quantitative sensory testing for mechanical somatosensory and pain modulation assessment of masticatory structures. J Oral Rehabil 2017;44:197-204.

30. Marcuzzi A, Wrigley PJ, Dean CM, Adams R, Hush JM. The long-term reliability of static and dynamic quantitative sensory testing in healthy individuals. Pain 2017;158:1217-1223.

31. Wolf OT. Stress and memory in humans: twelve years of progress? Brain Res 2009 13;1293:142-54.

32. Shields GS, Sazma MA, McCullough AM, Yonelinas AP. The effects of acute stress on episodic memory: A meta-analysis and integrative review. Psychol Bull 2017;143:636-675.

33. The American Institute of Stress. What is stress? https://www.stress.org/daily-life/. Acess in May 2017.

34. Chrousos GP. Stress and disorders of the stress system. Nat Rev Endocrinol 2009;5:374–381.

35. Koolhaas JM, Bartolomucci A, Buwalda B, de Boer SF, Flügge G, Korte SM, et al. Stress revisited: a critical evaluation of the stress concept. Neurosci Biobehav 2011;35:1291-301.

36. Schneiderman N, Ironson G, Siegel SD. Stress and health: Psychological, Behavioral, and Biological Determinants. Annu Rev Clin Psychol 2005;1:607-28.

37. Slade GD, Diatchenko L, Bhalang K, Sigurdsson A, Fillingim RB, Belfer I, et al. Influence of psychological factors on risk of temporomandibular disorders. J Dent Res 2007; 86:1120–1125.

38. Fillingim RB, Ohrbach R, Greenspan JD , Knott C, Diatchenko L, Dubner R , et al. Psychological Factors Associated with Development of TMD: the OPPERA Prospective Cohort Study. J Pain 2013;14:75-90.

39. Yatani H, Studts J, Cordova M, Carlson CR, Okeson JP. Comparison of sleep quality and clinical and psychologic characteristics in patients with temporomandibular disorders. J Orofac Pain 2002;16:221–228.

40. Schmitter M, Keller L, Giannakopoulos N, Rammelsberg P. Chronic stress in myofascial pain patients. Clin Oral Investig 2010;14:593-7.

41. Treede RD, Meyer RA, Raja SN, Campbell JN. Peripheral and central mechanisms of cutaneous hyperalgesia. Prog Neurobiol 1992;38: 397–421.

42. LaMotte RH, Thalhammer JG, Torebjork HE, Robinson CJ. Peripheral neural mechanisms of cutaneous hyperalgesia following mild injury by heat. J Neurosci 1982;2:765–81.

43. Baumann TK, Simone DA, Shain CN, LaMotte RH. Neurogenic hyperalgesia: the search for the primary cutaneous afferent fibers that contribute to capsaicin-induced pain and hyperalgesia. J Neurophysiol 1991;66:212–27.

44. Simone DA, Sorkin LS, Oh U, Chung JM, Owens C, LaMotte RH, et al. Neurogenic hyperalgesia: central neural correlates in responses of spinothalamic tract neurons. J Neurophysiol 1991;66:228–46.

45. Wallin M, Liedberg G, Börsbo B, Gerdle B.Thermal detection and pain thresholds but not pressure pain thresholds are correlated with psychological factors in women with chronic whiplash-associated pain. Clin J Pain 2012;28:211-21.

46. Hübscher M, Moloney N, Rebbeck T, Traeger A, Refshauge KM. Contributions of mood, pain catastrophizing, and cold hyperalgesia in acute and chronic low back pain: a comparison with pain-free controls. Clin J Pain 2014;30:886-93.

47. Tombaugh TN. A comprehensive review of the Paced Auditory Serial Addition Test (PASAT). Arch Clin Neuropsychol 2006;21:53–76

48. Tanosoto T, Arima T, Tomonaga A, Ohata N, Svensson P. A Paced Auditory Serial Addition Task evokes stress and differential effects on masseter-muscle activity and haemodynamics. Eur J Oral Sci 2012;120:363-7.

49. Tanosoto T, Bendixen KH, Arima T, Hansen J, Terkelsen AJ, Svensson P. Effects of the Paced Auditory Serial Addition Task (PASAT) with different rates on autonomic nervous system responses and self-reported levels of stress. J Oral Rehabil 2015;42:378-85.

50. Mathias CW, Stanford MS, Houston RJ. The physiological experience of the Paced Auditory Serial Addition Task (PASAT): Does the PASAT induce autonomic arousal? Arch Clin Neuropsychol 2004;19:543–554.

51. Girdler SS, Pedersen CA, Straneva PA, Leserman J, Stanwyck CL, Benjamin S, Light KC. Dysregulation of cardiovascular and neuroendocrine responses to stress in premenstrual dysphoric disorder. Psychiatry Res 1998; 81:163-78.

52. Pratt WM, Davidson D. Role of the HPA axis and the A118G polymorphism of the mu-opioid receptor in stress-induced drinking behavior. Alcohol Alcohol 2009;44:358-65.

53. Lustyk MK, Olson KC, Gerrish WG, Holder A, Widman L Psychophysiological and neuroendocrine responses to laboratory stressors in women: implications of menstrual cycle phase and stressor type. Biol Psychol 2010;83:84-92.

54. Maier C, Baron R, Tölle TR, Binder A, Birbaumer N, Birklein F, et al. Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): somatosensory abnormalities in 1236 patients with different neuropathic pain syndromes. Pain 2010;150:439–50.

55. Baron R, Maier C, Attal N, Binder A, Bouhassira D, Cruccu G, et al. Peripheral neuropathic pain: a mechanism-related organizing principle based on sensory profiles. Pain 2017;158:261-272.

56. Oka T. Psychogenic fever: how psychological stress affects body temperature in the clinical population. Temperature (Austin) 2015 3;2:368-78.

57. Gordon CJ. Thermal biology of the laboratory rat. Physiol Beh 1990; 47: 963–991.

58. Kiyatkin EA, Brown PL, Wise RA. Brain temperature fluctuation: a reflection of functional neural activation. Eur J Neurosci 2002;16:164–168.

59. Vianna DM, Carrive P. Changes in cutaneous and body temperature during and after conditioned fear to context in the rat. Eur J Neurosci 2005;21:2505-12.

60. Oka T, Oka K, Kobayashi T, Sugimoto Y, Ichikawa A, Ushikubi F, Narumiya S, Saper CB. Characteristics of thermoregulatory and febrile responses in mice deficient in prostaglandin EP1 and EP3 receptors. J Physiol 2003; 551:945-54;

61. Lkhagvasuren B, Nakamura Y, Oka T, Sudo N, Nakamura K. Social defeat stress induces hyperthermia through activation of thermoregulatory sympathetic premotor neurons in the medullary raphe region. Eur J Neurosci 2011; 34:1442-52;

62. Lkhagvasuren B, Oka T, Nakamura Y, Hayashi H, Sudo N, Nakamura K. Distribution of Fos-immunoreactive cells in rat forebrain and midbrain following social defeat stress and diazepam treatment. Neuroscience 2014; 272:34-57;

63. Diener SJ, Wessa M, Ridder S, Lang S, Diers M, Steil R, et al. Enhanced stress analgesia to a cognitively demanding task in patients with posttraumatic stress disorder. J Affect Dis 2012;136:1247–51.

64. Yilmaz P, Diers M, Diener S, Rance M, Wessa M, Flor H. Brain correlates of stress-induced analgesia. Pain 2010;151:522–9.

65. Vedolin GM, Lobato VV, Conti PC, Lauris JR. The impact of stress and anxiety on the pressure pain threshold of myofascial pain patients. J Oral Rehabil 2010;36:313-21.

66. Crettaz B, Marziniak M, Willeke P, Young P, Hellhammer D, Stumpf A, et al. Stress-induced allodynia - evidence of increased pain sensitivity in healthy humans and patients with chronic pain after experimentally induced psychosocial stress. Plos One 2013; 8:e69460.

67. Reinhardt T, Kleindienst N, Treede RD, Bohus M, Schmahl C. Individual modulation of pain sensitivity under stress. Pain Med 2013;14:676-85.

68. Levine FM, Krass SM, Padawar WJ. Failure hurts: the effects of stress due to difficult tasks and failure feedback on pain report. Pain 1993;54:335–40.

69. Caceres C; Burns JW. Cardiovascular reactivity to psychological stress may enhance subsequent pain sensitivity. Pain. 1997;69:237–244.

70. Wiech K, Kalisch R, Weiskopf N, Pleger B, Stephan KE, Dolan RJ. Anterolateral prefrontal cortex mediates the analgesic effect of expected and perceived control over pain. J Neurosci. 2006;26:11501–11509.

71. Naugle KM, Fillingim RB, Riley JL 3rd. A meta-analytic review of the hypoalgesic effects of exercise. J Pain 2012;13:1139-50.

72. Cathcart S, Winefield AH, Lushington K, Rolan P. Noxious inhibition of temporal summation is impaired in chronic tension-type headache. Headache 2010;50:403-12.

73. Geva N, Pruessner J, Defrin R. Acute psychosocial stress reduces pain modulation capabilities in healthy men. Pain 2014;155:2418-25.

74. Logan HL, Gedney JJ, Sheffield D, Xiang Y, Starrenburg E. Stress influences the level of negative affectivity after forehead cold pressor pain. J Pain. 2003;4:520–529.

75. Rhudy JL, Meagher MW. Fear and anxiety: divergent effects on human pain thresholds. Pain. 2000;84:65–75.

76. Rhudy JL, Meagher MW. Negative affect: effects on an evaluative measure of human pain. Pain 2003; 104:617-626.

77. Rhudy JL, Williams AE, McCabe KM, Russell JL, Maynard LJ. Emotional control of nociceptive reactions (ECON): do affective valence and arousal play a role? Pain 2008; 136: 250-261.

78. Jennings EM, Okine BN, Roche M, Finn DP. Stress-induced hyperalgesia. Prog Neurobiol 2014;121:1-18.

79. Marsland AL, Walsh C, Lockwood K, John-Henderson NA. The effects of acute psychological stress on circulating and stimulated inflammatory markers: A systematic review and meta-analysis. Brain Behav Immun 2017;12.

80. Donello JE, Guan Y, Tian M, Cheevers CV, Alcantara M, Cabrera S,et al. A peripheral adrenoceptor-mediated sympathetic mechanism can transform stress-induced analgesia into hyperalgesia. Anesthesiology 2011;114:1403-16.

81. Johnson AC, Greenwood-Van Meerveld B. Stress-induced pain: a target for the development of novel therapeutics. J Pharmacol Exp Ther 2014;351:327-35.

82. Lobbezoo F, Drangsholt M, Peck C, Sato H, Kopp S, Svensson P. Topical review: new insights into the pathology and diagnosis of disorders of the temporomandibular joint. J Orofac Pain 2004;18:181–91.

83. Woolf CJ. Central sensitization: implications for the diagnosis and treatment of pain. Pain 2011;152:S2–15.

84. Svensson P, Baad-Hansen L, Pigg M, List T, Eliav E, Ettlin D, et al. Special Interest Group of Oro-facial Pain. Guidelines and recommendations for assessment of somatosensory function in oro-facial pain conditions--a taskforce report. J Oral Rehabil 2011;38:366-94.

ANNEXES

ANNEX A – Stimulus modalities in QST and theirs distinct peripheral and central somatosensory pathways.

Type of stimulus	Physiological sensation elicited	Peripheral sensory channel	Central pathway
Mechanical			
Tactile	Touch	Aβ	Lemniscal
Vibration	Vibration	Aβ	Lemniscal
Brushing	Touch	Aδ, C	Lemniscal
Pinprick	Pinprick, sharp pain	Aδ, C	Spinothalamic
Deep pressure	Sharp pain	Aδ, C	Spinothalamic
Thermal			
Warm	Warmth	C	Spinothalamic
Cold	Cold	Aδ, C	Spinothalamic
Heat pain	Painful heat	Aδ, C	Spinothalamic
Cold pain	Painful cold	Aδ, C	Spinothalamic

Adapted from Svensson et al.[84] and Backonja et al[11].

ANNEX B – Guidelines for The Journal of Oral & Facial Pain and Headache:

Journal of Oral & Facial Pain and HEADACHE

Formerly *Journal of Orofacial Pain*

GUIDELINES FOR AUTHORS

Journal of Oral & Facial Pain and Headache is a quarterly journal that publishes scientifically sound articles of interest to practitioners and researchers in the field of pain, in particular orofacial pain and related conditions such as headache, temporomandibular disorders, and occlusally related disorders. The Journal publishes several types of peer-reviewed original articles:

1. ***Clinical and basic science research reports***—based on original research in pain, especially orofacial pain and related conditions. Case reports will also be considered provided they outline a background, well-documented clinical features (history, diagnostic and management approaches), and discussion of uncommon cases relevant to orofacial pain and related conditions.
2. ***Topical reviews***—dealing with a subject of relevance to pain, in particular orofacial pain and related conditions.
3a. ***Invited focus articles***—presenting a position or hypothesis on a basic science or clinical subject of relevance to orofacial pain and related conditions. These articles are not intended for the presentation of original results. Authors are selected by the Editorial Board.
3b. ***Invited commentaries***—critiquing a focus article by addressing the strong and weak points of the focus article. Authors of the commentaries are selected by the Editorial Board in consultation with the focus article author, and the focus article and the commentaries on it are published together in the Journal.
4. ***Proceedings of symposia, workshops, or conferences***—covering topics of relevance to orofacial pain and related conditions.

In addition, the Journal publishes:

5. ***Abstracts***—selected by the Editorial Board from those accepted by the AAOP or other affiliated academies. Criteria include originality and significance of findings, statistical basis of the data, conclusions appropriately drawn from the data, and appropriate grammatical expression.
6. ***Invited guest editorials***—may periodically be solicited by the Editorial Board.
7. ***Letters to the Editor***—may be submitted to the editor-in-chief; these should normally be no more than 500 words in length.
8. ***Literature abstracts***—abstracts of selected journal articles.
9. ***Meeting reviews***—highlights of selected scientific meetings.
10. ***Book reviews***—may periodically be solicited by the editorial board.

Review/editing of manuscripts. Manuscripts will normally be reviewed by the editor-in-chief, one associate editor, and at least two reviewers with expertise within the scope of the article. The publisher reserves the right to edit accepted manuscripts to ensure conciseness, clarity, and stylistic consistency, subject to the author's final approval.

Adherence to guidelines. Manuscripts not prepared in accordance with these guidelines or written in improper English will be returned with instructions to correct these problems prior to review.

MANUSCRIPT PREPARATION

The Journal will follow as much as possible the recommendations of the International Committee of Medical Journal Editors in regard to preparation of manuscripts and authorship (Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals; www.icmje.org/icmje-recommendations.pdf).

Manuscripts should be typed double-spaced with a 1-inch margin all around. Number all pages.

- **Title page.** This should include the title of the article (descriptive but as concise as possible) and the name, degrees, title, professional affiliation, and full address of all authors. Phone, fax, and email address must also be provided for the corresponding author, who will be assumed to be the first-listed author unless otherwise noted. If the paper was presented before an organized group, the name of the organization, location, and date should be included.
- **Abstract/keywords.** Include a maximum 250-word structured abstract (with headings Aims, Methods, Results, Conclusion) and five keywords.
- **Introduction.** Summarize the rationale and purpose of the study, giving only pertinent references. Clearly state the working hypothesis.
- **Materials and Methods.** Present materials and methods in sufficient detail to allow confirmation of the observations. Published methods should be referenced and discussed only briefly, unless modifications have been made. Indicate the statistical methods used, if applicable.
- **Results.** Present results in a logical sequence in the text, tables, and illustrations. Do not repeat in the text all the data in the tables or illustrations; emphasize only important observations.
- **Discussion.** Emphasize new and important aspects of the study and the conclusions that follow from them. Do not repeat in detail data or other material given in the Introduction or Results section. Relate observations to other relevant studies; point out the implications of the findings and their limitations.
- **Acknowledgments.** Acknowledge persons who have made substantive contributions to the study. Specify grant or other financial support, citing the name of the supporting organization and grant number.
- **Figure Legends.** Figure legends should be grouped at the end of the text and typed double-spaced.
- **Abbreviations.** The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.
- **Trade names.** Generic terms are to be used whenever possible, but trade names and manufacturer should be included parenthetically at first mention.

REFERENCES

- All references must be cited in the text, numbered in order of appearance.
- The reference list should appear at the end of the article in numeric sequence.
- Do not include unpublished data or personal communications in the reference list. Cite such references parenthetically in the text and include a date.
- Avoid using abstracts as references.
- Provide complete information for each reference, including names of all authors (up to six). If the reference is to part of a book, also include the title of the chapter and names of the book's editor(s).

Journal reference style:

1. Turp JC, Kowalski CJ, Stohler CS. Treatment-seeking patterns of facial pain patients: Many possibilities, limited satisfaction. J Orofac Pain 1998;12:61–66.

Book reference style:

1. Hannam AG, Langenbach GEJ, Peck CC. Computer simulations of jaw biomechanics. In: McNeill C (ed). Science and Practice of Occlusion. Chicago: Quintessence, 1997: 187–194.

ILLUSTRATIONS AND TABLES

- All illustrations and tables should be numbered and cited in the text in order of appearance.
- Illustrations and tables should be embedded in a PC Word or PDF document.
- All illustrations and tables should be grouped at the end of the text.
- Original artwork or slides may still be required of the author after acceptance of the article.

MANDATORY SUBMISSION FORM

The Mandatory Submission Form, signed by all authors, must accompany all submitted manuscripts before they can be reviewed for publication. This form can be downloaded from the Journal's website (www.manuscriptmanager.com/jop) under "Publisher's Home-Page." Please scan the completed form and email it to admin.jop@dentistry.utoronto.ca

PERMISSIONS AND WAIVERS

- Permission of author and publisher must be obtained for the direct use of material (text, photos, drawings) under copyright that does not belong to the author.
- Waivers must be obtained for photographs showing persons. When such waivers are not supplied, faces will be masked to prevent identification.
- Permissions and waivers should be emailed along with the Mandatory Submission Form to admin.jop@dentistry.utoronto.ca

REPRINTS

Reprints can be ordered from the publisher. The publisher does not stock reprints; however, back issues can be purchased.

Submit manuscripts via online submission service: **www.manuscriptmanager.com/jop**